FORMULATION DEVELOPMENT AND *IN-VITRO/EX-VIVO* EVALUATION OF NOVEL BUCCOADHESIVE FILMS OF METOPROLOL TARTRATE USING 2^3 FACTORIAL DESIGN TECHNIQUES

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Chennai - 600 032

In partial fulfillment for the award of Degree of

MASTER OF PHARMACY

(Pharmaceutics)

Submitted by

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MELMARUVATHUR - 603 319.

APRIL 2013

CERTIFICATE

This is to certify that the research work entitled **"FORMULATION DEVELOPMENT AND *IN-VITRO/EX-VIVO* EVALUATION OF NOVEL BUCCOADHESIVE FILMS OF METOPROLOL TARTRATE USING 2^3 FACTORIAL DESIGN TECHNIQUES"** Submitted to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, in partial fulfillment for the award of the Degree of the Master of Pharmacy was carried out by **BHARATHIRAJA. D (Register No. 26116003)** in the Department of Pharmaceutics under my direct guidance and supervision during the academic year 2012-2013.

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Dedicated

To

My Beloved

father ...✍

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BHARATHIRAJA.D

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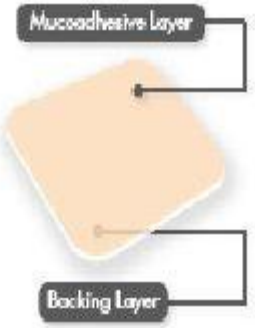
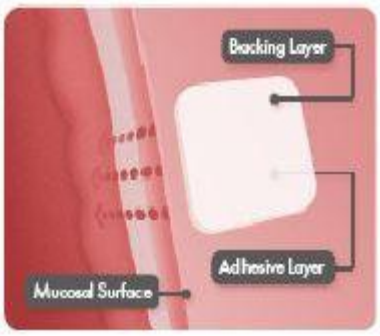
ABBREVIATIONS

HPMC	----	Hydroxy propyl methyl cellulose
CP	----	Carbopol
UV	----	Ultra Violet
µg	----	Microgram
λmax	----	Absorption maximum
mL	----	MilliLiter
N	----	Newton
mg	----	Milligram
FT-IR	----	Fourier Transform-Infra Red Spectroscopy
DSC	----	Differential Scanning Calorimetry
cm	----	Centimeter
%	----	Percentage
RH	----	Relative Humidity
USP	----	United State Pharmacopoeia
I P	----	Indian Pharmacopoeia
t	----	Time
ICH	----	International Conference on Harmonization
w/v	----	weight/volume
gm	----	Grams
RPM	----	Revolutions per Minute

mm	----	Millimeter
Sr. No.	----	Serial Number
Fig	----	Figure
°C	----	Degree Celsius
GIT	----	Gastrointestinal Tract
SD	----	Standard Deviation
eg	----	Example
Eq	----	Equation
%CDR	----	Percentage Cumulative Drug Release





INTRODUCTION

1. INTRODUCTION

1.1. Oral Drug Delivery System: - *(Chien Y. W., 2009; Shojaei A.H., 1998)*

For decades, per oral drug delivery has been the most widely utilized route of administration for the systemic delivery of drugs. The lack of efficacy of certain drugs due to decreased bioavailability, unpredictable and erratic absorption, GI intolerance or pre-systemic elimination has prompted the examination of other potential routes for administration. Moreover, the recent development of a large number of peptides as drugs has intensified investigation of mucosal delivery of drugs. Such routes exploring other absorptive mucosa include the oral, nasal, buccal, rectal, vaginal and ocular to a limited extend, pulmonary routes amongst the various route of drug delivery, oral route is perhaps the most preferred to the patient and the clinician alike. However, per oral administration of drugs has disadvantages such as hepatic first pass metabolism and enzymatic degradation within the GIT, that prohibit oral administration of certain classes of drugs especially peptides and proteins. Consequently, other absorptive mucosal are considered as potential sites for drug administration.

Trans mucosal route of drug delivery offer distinct advantages over peroral administration for systemic drug delivery. These advantages include possible bypass of first pass effect, avoidance of pre systemic elimination within the GIT and depending on the particular drug, a better enzymatic flora for drug absorption. The nasal cavity as a site for drug delivery has been investigated by many researchers and the route already reached commercial status with several drug including LHRH and calcitocin. However, the potential irritation and the reversible damage to the ciliary

action of the nasal cavity from chronic application of nasal dosage forms, as well as the large intra-and inter-subject variability in mucus secretion in the nasal mucosa, could significantly affect drug absorption from this site. Even though the rectal, vaginal and ocular mucosa all offer certain advantages, the poor patient acceptability associated with these sites renders them reserved for local application rather than systemic drug administration

As a site for drug delivery, the oral cavity offers several advantages over the gastrointestinal route and other alternative routes of drug administration. The membranes that line the oral cavity are readily accessible, robust and exhibit fast cellular recovery following local stress and damage. Oral mucosal drug delivery systems are easy and painless to administer and well accepted by the patient. Precise dosage form localization is possible and there is the ability to terminate delivery when required thus, patients could conceivably control the period of administration. For patient suffering with nausea or vomiting or in a state of unconsciousness with an upper GIT disease or surgery which affect oral drug absorption or those who have difficulty in swallowing per oral medications, the oral cavity may be useful site for drug delivery. The unique environment of the oral cavity dictates its potential as a site for drug delivery. The oral mucosa is highly perfused with blood vessels. It has a high blood flow of 20-50 mL/min. Because of the rich blood supply and direct access to the systemic circulation, the oral mucosal route is suitable for drugs which are susceptible to acid hydrolysis in the stomach or which are extensively metabolized in the liver. The continual secretion of saliva results in rapid removal of released drug and this may dictate that the oral cavity should be restricted to the delivery of drugs which have a short systemic action.

Conventional formulations for local oral delivery are principally lozenges, mouthwashes, mouth paints, oral gels, pastes and suspensions. Release of drugs from these preparations involves an initial burst of activity, whose level rapidly declines to sub-therapeutic concentrations. Retentive buccal mucoadhesive formulations may prove to be a viable alternative to the conventional oral medications as they can be readily attached to the buccal cavity, retained for a longer period of time and removed at any time. Attempts have been made earlier to formulate various mucoadhesive devices including Film, films, patches, disks, strips, ointments and gels. Buccal delivery of drugs provides an attractive alternative to the oral route of drug administration, particularly in overcoming deficiencies associated with the latter mode of dosing. Problems such as high first-pass metabolism and drug degradation in the harsh gastrointestinal environment can be circumvented by administering the drug via the buccal route. Moreover, buccal drug delivery offers a safer method of drug utilization, since drug absorption can be promptly terminated in cases of toxicity by removing the dosage form from the buccal cavity. It is also possible to administer drugs to patients who cannot be dosed orally via this route. Therefore, adhesive mucosal dosage forms were suggested for oral delivery, which included adhesive Film, adhesive gels and adhesive patches. A suitable buccal drug delivery system should be flexible and possess good bioadhesive properties, so that it can be retained in the oral cavity for the desired duration. In addition, it should release the drug in a controlled and predictable manner to elicit the required therapeutic response. Hydrogels are able to meet these requirements and they swell to a certain extent when placed in aqueous medium.

Of the range of pharmaceutical preparations available for administration into the oral cavity, the most popular form is that of a rapidly dissolving film that releases its drug contents for absorption across the oral mucosa. Alternatively, a film or capsule can be chewed to release its contents. This later method is less successful because mastication tends to produce a large volume of saliva that increases the probability of premature swallowing. The same problem occurs in the administration of drug in the form of a chewing gum.

1.2. Mucoadhesive Drug Delivery System:-

(Bhalodia R. et al., 2010; Gattani S. G. et al., 2006)

Mucoadhesive drug delivery system is a new system of drug delivery and has recently gained great concern in pharmaceutical sciences. The concept of mucoadhesives was introduced in the early 1980s. Residencecan be defined as the phenomenon of the attachment of natural or synthetic polymers to a mucosal surface. In general, the process involved in the Residence phenomenon can be described in three steps: first of all, the wetting and swelling of the polymer should allow an intimate contact with the tissue and secondly, interpenetration of the polymer chains and entanglement between the polymer and the mucin chains should be attained and finally, the formation of weak chemical bonds. Mucus is a viscous and heterogeneous biological product that coats many epithelial surfaces. Mucus-secreting cells are widely spread in different locations in the body, including the nasal, ocular, buccal area and the gastrointestinal, reproductive and respiratory tracts. Mainly, the mucus serves as a lubricant to minimize shear stresses and as a protection barrier against harmful substances. However, mucus can perform other important functions. Goblet cells located in the epithelium are unicellular mucus-secreting glands. Mucus is stored

in large granules in the goblet cell and can be released by exocytosis or exfoliation of the whole cell. Mucus granules are mainly stored in the apical side of the goblet cell, which results in the characteristic balloon shape of these cells. Although the secretion of mucus can vary depending on age, sex, body location and health condition, the average mucus turnover is approximately 6 h. Mucus consists mainly of water (up to 95% weight), inorganic salts (about 1% weight), carbohydrates and lipids (less than 1%) and glycoproteins (no more than 5% weight). Mucus glycolproteins are also called mucins and consist of a protein core with branched oligosaccharide chains attached over 63% of its length. Approximately 80% by weight of the glycoprotein consists of oligosaccharides, which make the mucin more hydrosoluble and also protects the protein core from proteolytic degradation.

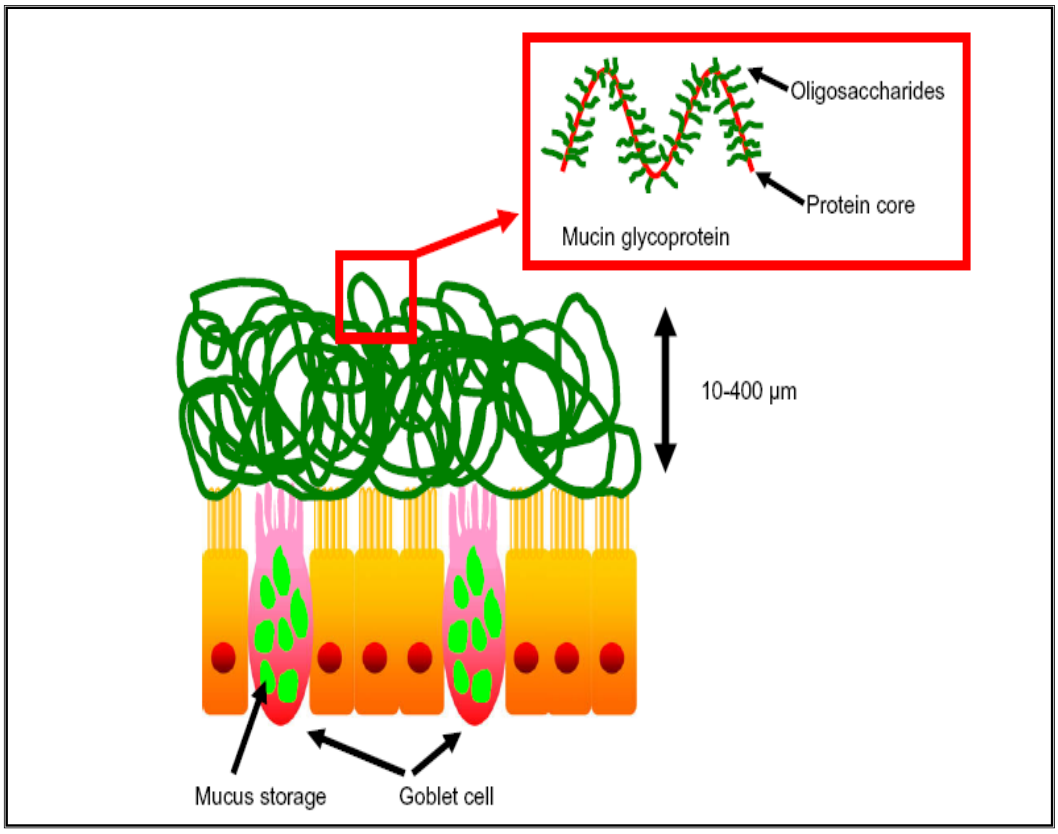


Fig. 1.1. Mucus layer on epithelial surface.

Mucus is a thin blanket covering all epithelia that are in contact with the external environment in the gastrointestinal, respiratory and urogenital tracts. In each case of these mucosal routes, mucus characteristics and functions are different. By this definition, the mucosal routes for drug delivery are:

1) Buccal drug delivery system
2) Nasal drug delivery system
3) Ocular drug delivery system
4) Vaginal drug delivery system
5) Gastrointestinal drug delivery system

1.2.1. Buccal Drug Delivery System:

(Prachiti P. V. et al., 2006; Rathbone M.J. et al., 1996; Vyas S. and Khar R. K., *2002)*

The oral cavity is viewed as a convenient and easily accessible site for the delivery of therapeutic agents. Within the oral cavity, drugs can be administered from the buccal gingiva or the sublingual space either for the treatment of local conditions (eg. thrush) or for the systemic treatment of diseases (eg. angina). The advances in bioadhesive and controlled release technology have stimulated a renewal of interest in the delivery of drugs to, or via the buccal route.

The buccal route of drug administration is the most widely used method for application of mucoadhesive delivery system. Both for the local treatment of infflamation and for rapid absorption of compounds, formulation technology have employed the buccal route for over two decades. The oral mucosa is covered by stratified squamous epithelium and three different types of mucosa can be distinguished: The masticatory, the lining and the specialized mucosa. Blood supply to the oral cavity tissues is delivered via the external carotid artery. The buccal

mucosa lines the inner cheek and buccal formulations are placed in the mouth between the upper gingivae (gums) and cheek (sometimes referred as buccal pouch) to treat local and systemic conditions.

Relative to the nasal and rectal routes, the buccal mucosa has low enzymatic activity and drug inactivation occurring biochemical degradation is not as rapid and extensive. The oral cavity consists of pair of buccal mucosa. Thus, a drug delivery system can be applied at various sites on the same mucosa or alternatively on the left or right buccal mucosa on different application. A buccal drug delivery system is applied to a specific area on the buccal membrane. Moreover the delivery system can be designed to the unidirectional in drug release, so that it can be protected from the local environment of the oral cavity. Within a oral mucosal cavity, delivery of drugs is classified into three categories:

1) Sublingual delivery – In this the systemic delivery of drugs through the mucosal membranes lining the floor of the mouth. This gives very fast onset of action of the drug but duration is usually short.
2) Buccal delivery – In this the drug administration through the mucosal membranes lining the cheeks (buccal mucosa) for buccal absorption, the buccal sulcus is used. This is the area between the upper lip and the gum. Film formulated for absorption from the buccal sulcus give a quick onset of action but will also give a longer duration of action than the sublingual route.
3) Local delivery – Which is the drug delivery into the oral cavity. The local delivery used for the treatment of toothache, periodontal diseases, dental caries, bacterial and fungal infections.

These sites differ from each other in terms of their anatomy and permeability. The sublingual mucosa is relatively permeable, giving rapid absorption and acceptable bioavailabilities of many drugs and is convenient, accessible and generally well accepted. The buccal membrane is sufficiently large to allow a delivery system to be placed at different sites on the same membrane on different occasions. This may be advantageous if the drug components of the delivery system or other excipients include in the formulation reversibly damage or irritate the mucosa. The oral cavity thus, on the other hand is highly acceptable by patients, the mucosa is relatively permeable with a rich blood supply, it is robust and shows short recovery times after stress or damage and the virtual lack of langerhans cells make the oral mucosa tolerant to potential allergens. These factors make the oral mucosal cavity a very attractive and feasible site for systemic drug delivery.

Table 1.1: Comparative properties of gastrointestinal, dermal and transmucosal drug administration

	Gastrointestinal	Dermal	Nasal	Oral mucosal	Vaginal
Accessibility	+	+++	++	++	+
Surface area	+++	+++	+	++	+++
Surface Enviornment	+	++	++	+++	+
Permiability	+++	+	+++	++	+++
Reactivity	++	++	+	+++	++
Vascular Drainage	+++	+	+++	++	+++
First pass clearance	+	+++	+++	+++	+
Patient acceptability	++	+++	++	+++	+++

+ Poor, + + Good, + + + Excellent

1.2.2. Advantages of Buccal Drug Delivery System: *(Bandyopadhyay A. K., 2008)*

1) Ease of administration and termination of therapy.
2) Permits localization of drugs to the oral cavity for prolonged period of time can be administered to unconscious patients.
3) There is relatively quick onset of action.
4) A significant reduction in dose can be achieved thereby reducing dose dependent side effects.
5) Presence of saliva facilitates both drug dissolution and its subsequent permeation by keeping the oral mucosa moist.
6) This route can be used for administration of drugs, which are unstable at acidic environment of the stomach or are destroy by the enzymatic flora.
7) The drug enters the general circulation without first passing through the liver.
8) Excellent accessibility to the buccal mucosa makes application of dosage form painless.
9) The delivery system can be designed to the unidirectional in drug release. So that it can be protected from the local environment of the oral cavity.
10) The buccal mucosa has low enzymatic activity and drug inactivation owing biochemical degradation is not as rapid and extensive.

1.2.3. Ideal Properties of Buccal Mucosal Drug Delivery:

(Bandyopadhyay A. K., 2008)

1) It should adhere to the site of attachment for a few hours.
2) It should release the drug in a controlled fashion.
3) It should provide drug release in an unidirectional way toward the mucosa.
4) It should facilitate the rate and extent of drug absorption.

5) It should not cause any irritation or inconvenience to the patient.
6) It should not interfere with the normal functions such as talking, drinking etc.

1.2.4. Limitations of Buccal Drug Delivery: *(Bhalodia R.et al., 2010)*

Drug administration via this route has certain limitations:

1) The surface area available for absorption in the buccal mucosa is much smaller than gastrointestinal, nasal, rectal and vaginal mucosae.
2) The buccal mucosa continuously bathed by saliva hence lowering the concentration of drug at absorbing site.
3) This route cannot administer drugs, which are unstable at buccal pH.
4) Drugs, which irritates buccal mucosa or have a bitter unpleasant taste or an obnoxious odour cannot administer by this route.
5) Because of the limited surface area, only a small dose can be administered.
6) Production of large volume of saliva increases the probability of premature swallowing.

1.3. Reported Mucoadhesive Buccal Dosage Forms:-

(Johnston T. P. et al., 2005; Sinko P.J., 2006)

Over the last 20 years a wide range of formulations has been developed for buccal drug delivery (Film, Discs, Patches, Gels, Ointments, Chewing gum and Mouthwashes) but comparatively few have found their way into the market. Buccal formulations have been developed to allow prolonged localized therapy and enhanced systemic delivery.

The most common formulations are Film and patches. Such formulations must be of a small size and a suitable geometry so as not to interfere with physiological function of the mouth, even after their hydration in the oral cavity. Moreover, in the

case of transmucosal administration, drug release should be unidirectional (towards the mucosa) and the release into the saliva should be avoided. Bioadhesive devices are broadly classified as,

1.3.1. Solid Buccal Adhesive Dosage Forms:-

1) Buccal Tablet: (*Gaurav Kumar Sharma et al.,2012)*

These are solid dosage forms prepared by the compression of powder mix that can be placed into contact with the oral mucosa and allow to adhere. They can deliver drug multidirectional into the oral cavity or to the mucosal surface. Alternatively, the presence of an impermeable layer can ensure that drug is delivered unidirectional. For systemic therapy, they will hold a drug in intimate contact with its absorbing surface, offer some protection to enzymatic degradation and avoid first pass metabolism. For local action, the formulation can be applied directly to a specific region. A typical bioadhesive formulation consists of a bioadhesive polymer (such as polyacrylic acids or a cellulose derivative) alone or in combination is incorporated into a matrix containing the active agent and excipients and perhaps the second layer to allow unidirectional drug delivery.

Table 1.2: Buccoadhesive Tablet containing bioadhesive polymers and Drugs

Sr. No.	Type of Formulation	Bioadhesives polymers	Drugs
1	Bilayer tablet	Sodium Alginate Carbopol 934 P, Ethyl Cellulose	Propranolol Hydrochloride
2	Matrix tablet	Methocel K4M, Carbopol 934 P, Ethyl Cellulose	Metaprolol Tartrate
3	Matrix tablet	Sodium CMC, HPMC, Carbopol 934 P	Prednisolone
4	Matrix tablet	Sodium CMC, HPMC K4M, Carbopol 934 P, HPMC K15M	Ondansetron Hydrochloride

2) Lozenges:

Bioadhesive lozenges may be used for the delivery of drugs that act topically within the mouth including antimicrobials, corticosteroids, local anesthetics, antibiotics and antifungal. Conventional lozenges produce a high initial release of drug in the oral cavity, which rapidly declines to sub therapeutic levels, thus multiple daily dosing is required. A slow release bioadhesive lozenge offers the potential for prolonged drug release with improved patient compliance. Codd and Deasy investigated bioadhesive lozenges as a means to deliver antifungal agents to the oral cavity.

1.3.2. Semi-Solid Buccal Adhesive Dosage Forms:-

These typically contain a bioadhesive polymers and drug plus any required excipients dissolved or suspended as a fine powder in an aqueous or non aqueous base, depending on their solubility and concentration. They can be applied by using the finger (or syringe) to a target region and tend to be more acceptable in terms of mouth fill to patients relative to a solid dosage form. However, they may deliver varying amount of active ingredients in comparison with a unit dosage form.

1) Films and Patches:

Patches are usually prepared by casting a solution of the polymer, drug and any excipients (such as plasticizer) on to a surface and allowing it to dry. Patches can be made $\leq$ 10-15 cm^2 in size but are more usually 1-3 cm^2 with perhaps an ellipsoid shape to fit comfortably into the centre of the buccal mucosa. In similar fashion to buccal Film, they can be made multidirectional or unidirectional. Patches are laminated and generally consist of an impermeable backing layer and a drug-containing layer that has mucoadhesive properties and from which the drug is released

in a controlled manner. Systems based on diclofenac, tannic and boric acids and intended for local administration have been developed. Due to relative thinness of the films, they are more susceptible to overhydration and loss of adhesive properties. Flexible films may be used to deliver drugs directly to a mucosalmembrane. They also offer advantages over creams and ointments in terms of delivery of measured dose of drug to the site. Buccal adhesive films are already available in market eg. Zilactin used for the therapy of canker sores, cold sores and lip sores.

Table 1.3: Semi-solid buccal adhesive dosage forms containing bioadhesive polymers and active agents.

Sr. No.	Type of formulation	Bioadhesives	Active agent
1	Film	Na CMC, PVP K-50	Diltiazem hydrochloride
2	Patch	Na CMC, HPMC K4M, Chitosan, HPMC K15M	Miconazole nitrate
3	Patch	Polycarbophil, Carbopol940, Xanthan gum	Benzydamine and Lidocaine

2) Gel:

Gel forming bioadhesive polymers include crosslinked polyacrylic acid that has been used to adhere to mucosal surfaces for extended period of time and provide controlled release of drug at the absorption site. A limitation of gel formulations lies on their inability to deliver a measured dose of drug to the site. They are therefore of limited use for drugs with narrow therapeutic window.

Table 1.4: Semi-solid buccaladhesive dosage forms containing bioadhesive polymers and active agents.

Sr. No.	Type of formulations	Bioadhesives	Active agent
1	Gel	Polycarbophil, PVP, HPMC	Chlorhexidine,Flubiprofen
2	Gel	Hexadimethrine	Triclosan
3	Ointment	Polymethacrylamide	Benzyl nicotinate

1.3.3. Liquids:-

Liquids have the advantage of being readily distributed throughout the oral cavity (eg. Mouth washes) but are not readily retained or targeted to the buccal mucosa and would deliver relatively uncontrolled amounts of an active ingredient. Viscous liquids may be used to coat buccal surface either as protectants or as drug vehicles for delivery to the mucosal surface. Traditionally, pharmaceutically acceptable polymers were used to enhance the viscosity of products to aid their retention in the oral cavity. Dry mouth is treated with artificial saliva solutions that are retained on mucosal surfaces to provide lubrication.

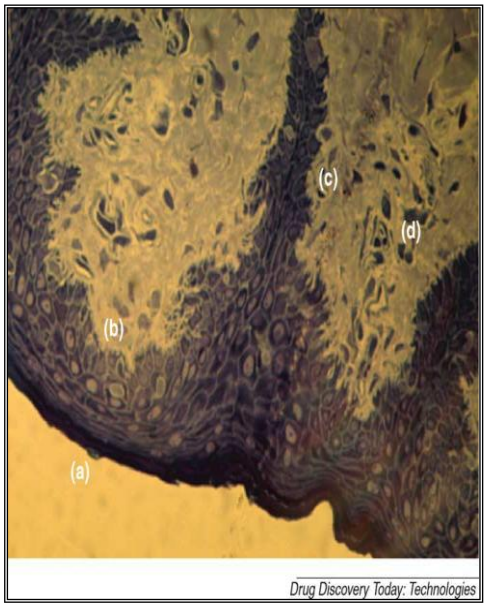
1.4. Overview of the Oral Mucosa: - *(Johnston T. P., 2005;Surendar Verma .,2011)*

Buccal region is that part of the mouth bounded anteriorly and laterally by the lips and the cheeks, posteriorly and medially by the teeth and/or gums, above and below by the reflections of the mucosa from the lips and cheeks to the gums. Numerous racemose, mucous or serous glands are present in the submucous tissue of the cheeks. The buccal glands are placed between the mucous membrane and

buccinator muscle: they are similar in structure to the labial glands, but smaller. About five of a larger size than the rest, are placed between the masseter and buccinators muscles around the distal extremity of the parotid duct; their ducts open in the mouth opposite the last molar tooth. They are called molar glands. Maxillary artery supplies blood to buccal mucosa and blood flow is faster and richer ($2.4ml/min/cm^2$) than that in the sublingual, gingival and palatal regions, thus facilitates passive diffusion of drug molecules across the mucosa.

The oral mucosa is composed of an outermost layer of stratified squamous epithelium (Fig. 1.3). Below this lies a basement membrane, a lamina propria followed by the submucosa as the innermost layer. The epithelium is similar to stratified squamous epithelia found in the rest of the body in that it has a mitotically active basal cell layer, advancing through a number of differentiating intermediate layers to the superficial layers, where cells are shed from the surface of the epithelium. The epithelium of the buccal mucosa is about 40-50 cell layers thick, while that of the sublingual epithelium contains somewhat fewer. The epithelial cells increase in size and become flatter as they travel from the basal layers to the superficial layers. The turnover time for the buccal epithelium has been estimated at 5-6 days, and this is probably representative of the oral mucosa as a whole. The oral mucosal thickness varies depending on the site: the buccal mucosa measures at 500-800 µm, while the mucosal thickness of the hard and soft palates, the floor of the mouth, the ventral tongue and the gingivae measure at about 100-200 µm. The composition of the epithelium also varies depending on the site in the oral cavity. The mucosa of areas subjected to mechanical stress (the gingivae and hard palate) are keratinized similar to the epidermis. The mucosa of the soft palate, the sublingual and

the buccal regions are not keratinized. The keratinized epithelia contain neutral lipids like ceramides and acylceramides which have been associated with the barrier function. These epithelia are relatively impermeable to water. In contrast, non-keratinized epithelia, such as the floor of the mouth and the buccal epithelia do not contain acylceramides and only have small amounts of ceramide. They also contain small amounts of neutral but polar lipids, mainly cholesterol sulfate and glucosyl ceramides. These epithelia have been found to be considerably more permeable to water than keratinized epithelia.



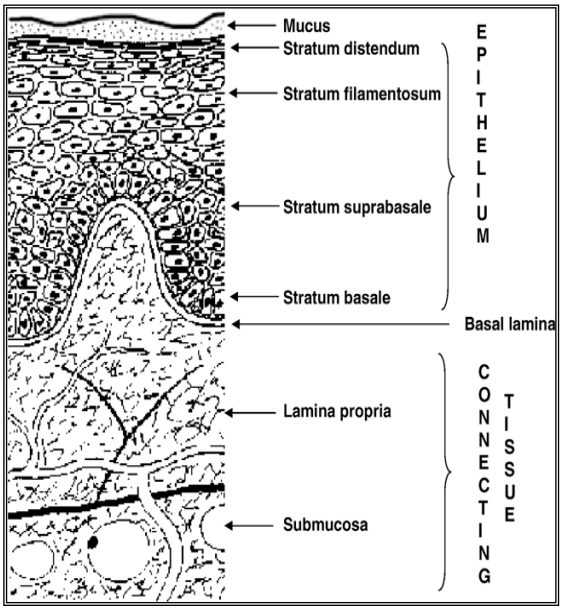


Fig 1.2 **Fig. 1.3**

Fig. 1.2: Section of the buccal epithelium. (a) Superficial layer; (b) basal layer; (c) Basal membrane and (d) lamina propria (underlying the connective tissue**)**

Fig. 1.3: Cross-section of buccal mucosa

The primary function of buccal epithelium is the protection of the underlying tissue. In nonkeratinized regions, lipid-based permeability barriers in the outer epithelial layers protect the underlying tissues against fluid loss and entry of

potentially harmful environmental agents such as antigens, carcinogens, microbial toxins and enzymes from foods and beverages.

1.4.1. Environment: *(Flavia Chiva carvalno et al.,2010)*

The oral cavity is marked by the presence of saliva produced by the salivary glands and mucus which is secreted by the major and minor salivary glands as part of saliva. The cells of the oral epithelia are surrounded by an intercellular ground substance, mucus, the principle components of which are complexes made up of proteins and carbohydrates. These complexes may be free of association or some maybe attached to certain regions on the cell surfaces. This matrix may actually play a role in cell-cell adhesion as well as acting as a lubricant, allowing cells to move relative to one another. Along the same lines, the mucus is also believed to play a role in bioadhesion of mucoadhesive drug delivery systems. In stratified squamous epithelia found elsewhere in the body, mucus is synthesized by specialized mucus secreting cells like the goblet cells, however in the oral mucosa; mucus is secreted by the major and minor salivary glands as part of saliva. Up to 70% of the total mucin found in saliva is contributed by the minor salivary glands. At physiological pH the mucus network carries a negative charge (due to the sialic acid and sulfate residues) which may play a role in mucoadhesion. At this pH mucus can form a strongly cohesive gel structure that will bind to the epithelial cell surface as a gelatinous layer.

- **Role of Mucus:**
  - Made up of proteins and carbohydrates
  - Cell-cell adhesion
  - Lubrication
  - Bioadhesion of mucoadhesive drug delivery systems

Another feature of the environment of the oral cavity is the presence of saliva produced by the salivary glands. Saliva is the protective fluid for all tissues of the oral cavity. It protects the soft tissues from abrasion by rough materials and from chemicals. It allows for the continuous mineralization of the tooth enamel after eruption and helps in demineralization of the enamel in the early stages of dental caries. Saliva is an aqueous fluid with 1% organic and inorganic materials. The major determinant of the salivary composition is the flow rate which in turn depends upon three factors: the time of day, the type of stimulus, and the degree of stimulation. The salivary pH ranges from 5.5 to 7 depending on the flow rate. At high flow rates, the sodium and bicarbonate concentrations increase leading to an increase in the pH. The daily salivary volume is between 0.5 to 2 liters and it is the amount of fluid that is available to hydrate oral mucosal dosage forms. A main reason behind the selection of hydrophilic polymeric matrices as vehicles for oral transmucosal drug delivery systems is this water rich environment of the oral cavity.

- **Role of Saliva:**
  - Protective fluid for all tissues of the oral cavity
  - Continuous mineralization / demineralization of the tooth enamel
  - To hydrate oral mucosal dosage forms

1.4.2. Permeability: *(Bhalodia R. et al., 2010;Ravi Saurabh et al.,2011)*

The oral mucosa, in general is a somewhat leaky epithelia intermediate between that of the epidermis and intestinal mucosa. It is estimated that the permeability of the buccal mucosa is 4-4000 times greater than that of the skin. As indicative by the wide range in this reported value, there are considerable differences in permeability between different regions of the oral cavity because of the diverse

structures and functions of the different oral mucosae. In general, the permeabilities of the oral mucosae decrease in the order of sublingual greater than buccal and buccal greater than palatal. This rank order is based on the relative thickness and degree of keratinization of these tissues with the sublingual mucosa being relatively thin and non-keratinized, the buccal thicker and non-keratinized and the palatal intermediate in thickness but keratinized.

It is currently believed that the permeability barrier in the oral mucosa is a result of intercellular material derived from the so-called 'membrane coating granules' (MCG). When cells go through differentiation, MCGs start forming and at the apical cell surfaces, they fuse with the plasma membrane and their contents are discharged into the intercellular spaces at the upper one third of the epithelium. This barrier exists in the outermost 200μm of the superficial layer. Permeation studies have been performed using a number of very large molecular weight tracers, such as horseradish peroxidase and lanthanum nitrate. When applied to the outer surface of the epithelium, these tracers penetrate only through outermost layer or two of cells. When applied to the submucosal surface, they permeate up to but not into the outermost cell layers of the epithelium. According to these results, it seems apparent that flattened surface cell layers present the main barrier to permeation, while the more isodiametric cell layers are relatively permeable. In both keratinized and non-keratinized epithelia, the limit of penetration coincided with the level where the MCGs could be seen adjacent to the superficial plasma membranes of the epithelial cells. Since, the same result was obtained in both keratinized and non-keratinized epithelia; keratinization by itself is not expected to play a significant role in the barrier function. The components of the MCGs in keratinized and non-keratinized epithelia

are different. However, the MCGs of keratinized epithelium are composed of lamellar lipid stacks whereas; the non-keratinized epithelium contains MCGs that are non-lamellar. The MCG lipids of keratinized epithelia include sphingomyelin, glucosylceramides, ceramides and other nonpolar lipids. However, for non-keratinized epithelia, the major MCG lipid components are cholesterol esters, cholesterol and glycosphingolipids. Aside from the MCGs, the basement membrane may present some resistance to permeation as well, however the outer epithelium is still considered to be the rate limiting step to mucosal penetration. The structure of the basement membrane is not dense enough to exclude even relatively large molecules.

1.5. Buccal Absorption:-

1.5.1. Buccal Routes of Drug Absorption:

(Rathbone M.J.et al., 1996;Sachin Shankar et al.,2012)

There are two permeation pathways for passive drug transport across the oral mucosa: paracellular and transcellular routes. Permeants can use these two routes simultaneously, but one route is usually preferred over the other depending on the physicochemical properties of the diffusant. Since, the intercellular spaces and cytoplasm are hydrophilic in nature; lipophilic compounds would have low solubilities in this environment. The cell membrane, however, is rather lipophilic in nature and hydrophilic solutes will have difficulty permeating through the cell membrane due to a low partition coefficient. Therefore, the intercellular spaces pose as the major barrier to permeation of lipophilic compounds and the cell membrane acts as the major transport barrier for hydrophilic compounds. Since, the oral epithelium is stratified; solute permeation may involve a combination of these two

routes. The route that predominates, however, is generally the one that provides the least amount of hindrance to passage.

- ❖ Passive diffusion
  - ➢ Transcellular or intracellular route (crossing the cell membrane and entering the cell)
  - ➢ Paracellular or intercellular route (passing between the cells)

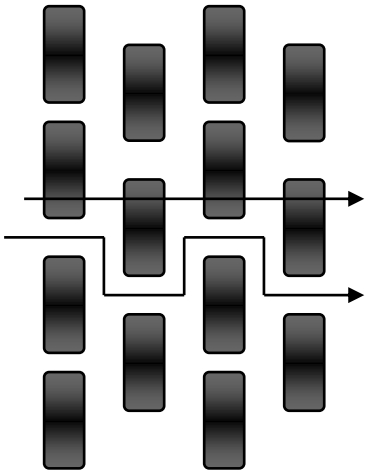


Fig. 1.4: Mechanism of transmucosal permeation.

- ❖ Carrier mediated transport
- ❖ Endocytosis

The flux of drug through the membrane under sink condition for paracellular route can be written as Eq. (1)

$$J_p = \frac{D_p \varepsilon}{h_p} C_d$$ ----------Equation 1

Where,

D_p is diffusion coefficient of the permeate in the intercellular spaces

h_p is the path length of the paracellular route

ε is the area fraction of the paracellular route and

C_d is the donor drug concentration

Similarly, flux of drug through the membrane under sink condition for transcellular route can be written as Eq. (2).

$$J_c = \frac{(1-\varepsilon)D_c K_c}{h_c} C_d$$ ----------Equation 2

Where,

K_c is partition coefficient between lipophilic cell membrane and the aqueous phase

D_c is the diffusion coefficient of the drug in the transcellular spaces and

h_c is the path length of the transcellular route.

In very few cases absorption also takes place by the process of endocytosis where the drug molecules were engulfed by the cells. It is unlikely that active transport processes operate within the oral mucosa; however, it is believed that acidic stimulation of the salivary glands with the accompanying vasodilatation facilitates absorption and uptake into the circulatory system. The absorption potential of the buccal mucosa is influenced by the lipid solubility and molecular weight of the diffusant. Absorption of some drugs via the buccal mucosa is found to increase when carrier pH is lowered and decreased with an increase of pH. However, the pH dependency that is evident in absorption of ionizable compounds reflects their partitioning into the epithelial cell membrane, so it is likely that such compounds will tend to penetrate transcellularly. Weak acids and weak bases are subjected to pH-dependent ionization. It is presumed that ionized species penetrate poorly through the oral mucosa compared with non-ionized species. An increase in the amount of non-

ionized drug is likely to increase the permeability of the drug across an epithelial barrier and this may be achieved by a change of pH of the drug delivery system. It has been reported that pH has effect on the buccal permeation of drug through oral mucosa. The diffusion of drugs across buccal mucosa was not related to their degree of ionization as calculated from the Henderson–Hasselbalch equation and thus it is not helpful in the prediction of membrane diffusion of weak acidic and basic drugs.

1.5.2. Buccal Mucosa as a Site for Drug Delivery:

(Bhalodia R. et al., 2010*;Chinna Reddy P et al.,2011)*

There are three different categories of drug delivery within the oral cavity (i.e., sublingual, buccal, and local drug delivery). Selecting one over another is mainly based on anatomical and permeability differences that exist among the various oral mucosal sites. The sublingual mucosa is relatively permeable, giving rapid absorption and acceptable bioavailabilities of many drugs. It is convenient, accessible and generally well accepted. Even though the sublingual mucosa is relatively more permeable than the buccal mucosa, it is not suitable for an oral transmucosal delivery system. The sublingual region lacks an expanse of smooth muscle or immobile mucosa and is constantly washed by a considerable amount of saliva making it difficult for device placement. Because of the high permeability and the rich blood supply, the sublingual route is capable of producing a rapid onset of action making it appropriate for drugs with short delivery period requirements with infrequent dosing regimen. Due to two important differences between the sublingual mucosa and the buccal mucosa, the latter is a more preferred route for systemic transmucosal drug delivery. First difference is in the permeability characteristics of the region, where the buccal mucosa is less permeable and is thus not able to give a rapid onset of

absorption (i.e., more suitable for a sustained release formulation) and second is that, the buccal mucosa has an expanse of smooth muscle and relatively immobile mucosa which makes it a more desirable region for retentive systems used for oral transmucosal drug delivery. Thus, the buccal mucosa is more fitted for sustained delivery applications, delivery of less permeable molecules, and perhaps peptide drugs.

1.5.3 Factors Influencing Buccal Absorption:

(Brahmankar D.M. and Jaiswal S.B., *2006)*

Factors that affect the buccal absorption are;

1) Biphasic solubility of drug :

The drug should be sufficient lipophillic to cross the oral mucosal barrier and sufficiently hydrophilic to dissolute in saliva. Both lipophilicity and hydrophilicity i.e. biphasic solubility of the drug is required for buccal/sublingual absorption of drug.

2) Salivary secretion:

For absorption through oral mucosa, the drug must be soluble in the aqueous buccal fluid. The absorption also depends on the secretion of saliva. Absorption is retarded if the mouth is dry.

3) pH of saliva:

The salivary pH ranges between 6 and 7. Increasing the pH of fluid in buccal cavity promotes absorption of the weak base but retarded the absorption of weak acid.

4) Temperature:

Temperature also affects the buccal absorption of drug to a lesser extent.

5) Binding to oral mucosa:

The systemic availability of the drugs that bind to the oral mucosa is poor.

1.6. Experimental Methodology for Buccal Permeation Studies: -

(Chinna Reddy P et al.,2011.,Shojaei A.H., 1998)

Before a buccal drug delivery system can be formulated, buccal absorption/permeation studies must be conducted to determine the feasibility of this route of administration for the drug. These studies involve methods that would examine *In-vitro* and/or *in-vivo* buccal permeation profile and absorption kinetics of the drug.

1.6.1. *In-vitro* Methods:

At the present time, most of the *In-vitro* studies examining drug transport across buccal mucosa have used buccal tissues from animal models. Animals are sacrificed immediately before the start of an experiment. Buccal mucosa with underlying connective tissue is surgically removed from the oral cavity, the connective tissue is then carefully removed and the buccal mucosal membrane is isolated. The membranes are then placed and stored in ice-cold (4 °C) buffers (usually Krebs buffer) until mounted between side-by-side diffusion cells for the *In-vitro* permeation experiments. The most significant questions concerning the use of animal tissues as *In-vitro* models in this manner are the viability and the integrity of the dissected tissue. How well the dissected tissue is preserved is an important issue which will directly affect the results and conclusion of the studies. To date, there are no standard means by which the viability or the integrity of the dissected tissue can be assessed and studied tissue viability by using ATP levels in rabbit buccal mucosa. Using ATP levels as an indicator for tissue viability is not necessarily an accurate measure. A 50% drop in the tissue ATP concentration during the initial 6 hours of the experiment without a corresponding drop in tissue permeability. Despite certain

gradual changes, the buccal tissue seems to remain viable for a rather long period of time. Therefore, a decrease in ATP levels does not assure a drop in permeability characteristics of the tissue. The most meaningful method to assess tissue viability is the actual permeation experiment itself, if the drug permeability does not change during the time course of the study under the specific experimental conditions of pH and temperature, then the tissue is considered viable.

Buccal cell cultures have also been suggested as useful *In-vitro* models for buccal drug permeation and metabolism. However, to utilize these culture cells for buccal drug transport, the number of differentiated cell layers and the lipid composition of the barrier layers must be well characterized and controlled. This has not yet been achieved with the buccal cell cultures used so far.

1.6.2. *In- vivo* Methods:

In-vivo methods were first originated by Beckett and Triggs with the so-called buccal absorption test. Using this method, the kinetics of drug absorption was measured. The methodology involves the swirling of a 25 ml sample of the test solution for up to 15 minutes by human volunteers followed by the expulsion of the solution. The amount of drug remaining in the expelled volume is then determined in order to assess the amount of drug absorbed. The drawbacks of this method include salivary dilution of the drug, accidental swallowing of a portion of the sample solution and the inability to localize the drug solution within a specific site (buccal, sublingual or gingival) of the oral cavity. Various modifications of the buccal absorption test have been carried out correcting for salivary dilution and accidental swallowing, but these modifications also suffer from the inability of site localization. A feasible approach to achieve absorption site localization is to retain the drug on the buccal

mucosa using a bioadhesive system. Pharmacokinetic parameters such as bioavailability can then be calculated from the plasma concentration vs. time profile.

Other *in-vivo* methods include a small perfusion chamber attached to the upper lip of anesthetized dogs. The perfusion chamber is attached to the tissue by cyanoacrylate cement. The drug solution is circulated through the device for a predetermined period of time and sample fractions are then collected from the perfusion chamber (to determine the amount of drug remaining in the chamber) and blood samples are drawn after 0 and 50 minutes (to determine amount of drug absorbed across the mucosa).

1.7. Buccal Drug Delivery and Mucoadhesivity:-

(Shojaei A.H., 1998; Gattani S. G. et al., 2006)

In the development of these buccal drug delivery systems, Residence of the device is a key element. The term 'mucoadhesive' is commonly used for materials that bind to the mucin layer of a biological membrane. Mucoadhesive polymers have been utilized in many different dosage forms in efforts to achieve systemic delivery of drugs through the different mucosa. These dosage forms include Film, patches, tapes, films, semisolids and powders. To serve as mucoadhesive polymers, the polymers should possess some general physiochemical features such as.

i.Predominantly anionic hydrophilicity with numerous hydrogen bond-forming groups

ii. Suitable surface property for wetting mucus/mucosal tissue surfaces and

iii. Sufficient flexibility to penetrate the mucus network or tissue crevices.

Following polymers have been tried and tested over the year that includes:

Table 1.5: Recent Research on Mucoadhesive Polymers and Its Delivery Systems:

Bioadhesive Polymer(s) Studied	Investigation Objectives
HPC and CP	Preferred mucoadhesive strength on CP, HPC, and HPC-CP combination
HPC and CP	Measured Bioadhesive property using mouse peritoneal membrane
CP, HPC, PVP, CMC	Studied inter polymer complexation and its effects on bioadhesive strength
CP and HPMC	Formulation and evaluation of buccoadhesive controlled release delivery systems
HPC, HEC, PVP, and PVA	Tested mucosal adhesion on patches with two-ply laminates with an impermeable backing layer and hydrocolloid polymer layer
HPC and CP	Used HPC-CP powder mixture as peripheral base for strong adhesion and HPC-CP freeze dried mixture as core base
CP, PIP, and PIB	Used a two roll milling method to prepare a new bioadhesive patch formulation
Xanthum gum and Locust bean gum	Hydrogel formation by combination of natural gums
Chitosan, HPC, CMC, Pectin, Xantham gum, and Polycarbophil	Evaluate mucoadhesive properties by routinely measuring the detachment force form pig intestinal mucosa
Hyaluronic acid benzyl esters, Polycarbophil, and HPMC	Evaluate mucoadhesive properties
Hydroxyethylcellulose	Design and synthesis of a bilayer patch (polytef-disk) for thyroid gland diagnosis
Polycarbophil	Design of a unidirectional buccal patch for oral mucosal delivery of peptide drugs
Poly(acrylic acid) and Poly(methacrylic acid)	Synthesized and evaluated crosslinked polymers differing in charge densities and hydrophobicity
Number of Polymers including HPC, HPMC, CP, CMC.	Measurement of bioadhesive potential and to derive meaningful information on the structural requirement for bioadhesion

Poly(acrylic acid-co-acrylamide)	Adhesion strength to the gastric mucus layer as a function of crosslinking agent, degree of swelling, and carboxyl group density
Poly(acrylic acid)	Effects of PAA molecular weight and crosslinking concentration on swelling and drug release characteristics
Poly(acrylic acid-co-methyl methacrylate)	Effects of polymer structural features on mucoadhesion
Poly(acrylic acid-co- butylacrylate)	Relationships between structure and adhesion for mucoadhesive polymers
HEMA copolymerized with Polymeg® (polytetramethylene glycol)	Bioadhesive buccal hydrogel for controlled release delivery of buprenorphine
Cydot® by 3M (bioadhesive polymeric blend of CP and PIB)	Patch system for buccal mucoadhesive drug delivery
Formulation consisting of PVP, CP, and cetylpyridinium chloride (as stabilizer)	Device for oramucosal delivery of LHRH - device containing a fast release and a slow release layer
CMC, Carbopol 974P, Carbopol EX-55, Pectin (low viscosity), Chitosan chloride,	Mucoadhesive gels for intraoral delivery
CMC, CP, Polyethylene oxide, Polymethylvinylether/Maleic anhydride (PME/MA).	Buccal mucoadhesive device for controlled release anticandidal device - CMC Film yielded the highest adhesive force
HPMC and Polycarbophil (PC)	Buccal mucoadhesive Film with optimum blend ratio of 80:20 PC to HPMC yielding the highest force of adhesion
PVP, Poly(acrylic acid)	Transmucosal controlled delivery of isosorbide dinitrate

Poly(acrylic acid-co-poly ethyleneglycol) copolymer of acrylic acid and poly ethyleneglycol monomethylether monomethacryalte	To enhance the mucoadhesive properties of PAA for buccal mucoadhesive drug delivery
Poly acrylic acid and poly ethylene glycol	To enhance mucoadhesive properties of PAA by interpolymer complexation through template polymerization
Drum dried waxy maize starch (DDWM), Carbopol 974P, and sodium stearylfumarate	Bioadhesive erodible buccal film for progesterone delivery

1.8. Buccal Adhesive Polymers: - *(Chien Y. W., 2009; Johnston T. P. et al., 2005)*

Polymer is a generic term used to describe a very long molecule consisting of structural units and repeating units connected by covalent chemical bonds. The key feature that distinguishes polymers from other molecules is the repetition of many identical, similar, or complementary molecular subunits in these chains. These subunits, the monomers, are small molecules of low to moderate molecular weight and are linked to each other during a chemical reaction called polymerization. Instead of being identical, similar monomers can have varying chemical substituents. The differences between monomers can affect properties such as solubility, flexibility and strength. The term buccal adhesive polymer covers a large, diverse group of molecules, including substances from natural origin to biodegradable grafted copolymers and thiolated polymers. Bioadhesive formulations use polymers as the adhesive component. These formulations are often water soluble and when in a dry form attract water from the biological surface and this water transfer leads to a strong interaction. These polymers also form viscous liquids when hydrated with water that

increases their retention time over mucosal surfaces and may lead to adhesive interactions. Bioadhesive polymers should possess certain physicochemical features including hydrophilicity, numerous hydrogen bond-forming groups, flexibility for interpenetration with mucus, epithelial tissue and visco-elastic properties.

1.8.1. Ideal Characteristics:

(Saroj Kumar R. and Bala P.*, 2010; Bandyopadhyay A. K., 2008)*

- Polymer and its degradation products should be non-toxic, non-irritant and free from leachable impurities.
- It should have good spreadability, wetting and swelling properties, solubility and biodegradability.
- The pH should be biocompatible and should possess good viscoelastic properties.
- It should adhere quickly to buccal mucosa and should possess sufficient mechanical strength.
- It should possess peel, tensile and shear strengths at the bioadhesive range.
- Polymer must be easily available and its cost should not be high.
- It should show bioadhesive properties in both dry and liquid state.
- It should demonstrate local enzyme inhibition and penetration enhancement properties.
- It should demonstrate acceptable shelf life.
- It should have optimum molecular weight.
- It should possess active groups responsible for adhesion.
- It should have required spatial conformation.

- It should be sufficiently cross-linked but not to the degree of suppression of bond forming groups.
- It should not aid in development of secondary infections such as dental caries.

1.9. Commercial Buccal Adhesive Drug Delivery Systems:

(Sudhakar Y. et al., 2006)

Recent reports suggested that the market share of buccal adhesive drug delivery systems are increasing in the American and European market with the steady growth rate of above 10%. Some of the commercially available buccal adhesive formulations are listed in Table 1.6.

Table 1.6: Buccal formulations marketed or in clinical trial intended for both mucosal (local), or transmucosal (systemic) Administration

Sr. N0.	Brand Name	Active agent	Effect	Functional agent	Company
1	Aphtach (Tab)	Triamcinolone acetonide	Local (mouth)	HPC, Polyacrylic acid	Teijin Ltd
2	Buccastem (Tab)	Prochlorperazine	Systemic	Xanthan gum, Povidone	Reckitt Benkiser Plc
3	Oralin-Generex (Soln)	Insulin	Systemic	Unknown	Generex Biotechnoloy
4	Lauriad (Tab)	Miconazole	Local (mouth & Oropharyx)	Unknown	BioAlliance Pharma
5	Striant SR (Tab)	Testosterone	Systemic	HPMC, Carbomer 934 P	Ardama Bioscience Ltd.

LITERATURE SURVEY

2. LITERATURE SURVEY

2.1. Literature Review:-

Recent Advancements in Bucccoadhesive Drug Delivery Systems:

1) **Siddarth S. D. and Upendra K., (2010)** formulated a buccal dosage form. A number of buccal mucoadhesive patches of Felodipine were prepared by casting method using polyvinyl pyrrolidone (PVP) and polyvinyl alcohol (PVA) as polymer. Glycerin and propylene glycol were used as plasticizers, Stability study revealed that the percent drug content decreased in various patches was ranging from 1.15 to 1.90.

2) **Saroj Kumar R. and Bala P., (2010)** had described**,** of the various routes of drug delivery; the oral route is often preferred by the patient. However, peroral administration of drugs has disadvantages such as hepatic first-pass metabolism and enzymatic degradation within the gastrointestinal tract which constitutes a hindrance to oral administration of certain classes of drugs, especially peptides and proteins. This review describes various bio/mucoadhesive polymers used in transmucosal drug delivery.

3) **Subash P. *et al*., (2010)** had formulated Buccal patches of Isoxsuprine Hydrochloride, a potent and long acting vasodilator and uterine suppressant , by using Hydroxyl propyl methyl cellulose(HPMC), Polyvinyl pyrolidone K-50

(PVP K-50) and Hydroxyl ethyl cellulose (HEC). Higuchis plot studies revealed that the predominant mechanism of drug release was diffusion.

4) **Navneet G. *et al*., (2010)** had prepared Mucoadhesive Tablets of Salbutamol Sulphate by non aqueous granulation of polymers HPMC K-4M (Hydroxypropyl Methyl Cellulose) & EC (Ethyl Cellulose) in different ratios 1:1. 1:2 & 2:1. In vitro bioadhesive strength studies showed that Film containing more HPMC K-4M were great bioadhesive in nature. The maximum in-vitro release observed in formulation HE-1. (1:1 ratio) and the kinetics studies shows that release follows peppas model.

5) **Asha S. J. *et al*., (2010)** studied mucoadhesive bilayer buccal tablets of Atorvastatin Calcium using the bioadhesive polymers Carbopol 934P (CP), Sodium CMC, Hydroxy ethyl cellulose (HEC) and Sodium alginate (Na-alginate) along with ethyl cellulose as an impermeable backing layer. Film containing CP and Na-CMC in the ratio of 3:2 (F2) had the maximum percentage of *in-vitro* drug release without disintegration in 6 h.

6) **Hirlekar R. S., (2009)** prepared Carvedilol buccal tablet, Drug-Methyl-β-cyclodextrin complex was prepared by kneading method and characterized by Fourier Transformation Infrared spectroscopy, Differential Scanning Calorimetry and powder X-Ray Diffractometry studies.

7) **Rajesh S. P. and Poddar S. S., (2009)** had prepared and evaluated of mucoadhesive buccal patches for the controlled systemic delivery of Salbutamol Sulphate to avoid first pass hepatic metabolism. The developed

patches were evaluated for the physicochemical, mechanical and drug release characteristics. The patches showed desired mechanical and physicochemical properties to withstand environment of oral cavity.

8) **Aleksandra M. S. *et al.*, (2008)** had determine the possibility of transmucosal iontophoretic delivery of cationic drug and to investigate *ex vivo* Galantamine HBr and Naltrexone Hydrochloride administration via buccal mucosa by applying the iontophoresis and to define of initial donor drug concentration (in the presence and without of competitive cations) and current density influences on drug flux.

9) **Thimmasetty J. *et al.*, (2008)** had prepared Carvedilol patches using HPMC, carbopol 934, eudragit RS 100, and ethyl cellulose. The patches were evaluated for their thickness uniformity, folding endurance, weight uniformity, content uniformity, swelling behaviour, tensile strength, and surface pH. *In vitro* release studies were conducted for carvedilol-loaded patches. *In vivo* drug release studies in rabbits showed 90.85% of drug release from HPMC-carbopol patch while it was 74.63 to 88.02% within 90 min in human volunteers. Good correlation among *in vitro* release and *in vivo* release of carvedilol was observed.

10) **Akpa P. A. *et al.*, (2008)** studied the buccoadhesive and *in vitro* release properties of patches formulated with ethyl cellulose (EC) and hydroxyl propyl methyl cellulose (HPMC) interpolymer complexes of different ratios. The result of the study indicated that1:2 ratios of EC and HPMC gave the highest buccoadhesive strength.

11) **Belgamwar V. S. *et al.*, (2007)** prepared mucoadhesive multiparticulate system following ionic gelatin technique for oral drug delivery. Microspheres so prepared showed encapsulation efficiency ranging between 60% - 70% and had a mean particle size 400- 700µm as determined by optical microscopy.

12) **Madhusudan R. Y. *et al.*, (2007)** had developed a buccal patch for systemic administration of Carvedilol in the oral cavity has been using two different mucoadhesive polymers. The results indicate that suitable bioadhesive buccal patches with desired permeability could be prepared. . The bioavailability of carvedilol from buccal patches has increased 2.29 folds when compared to that of oral solution. The formulation AC5 (HPMC E 15) shows 84.85 + 0.089% release and 38.69 + 6.61% permeated through porcine buccal membrane in 4 hr.

13) **Ramana M. V. *et al.*, (2007)** fabricated mucoadhesive buccal tablet of Metaprolol Tartarate with objective of avoiding first pass metabolism and providing duration of action. The best mucoadhesive performance and in-vitro drug release profile were exhibited by the Film containing hydroxyl ethyl cellulose and carbopol-934 in ratio 1:2.

14) **Nakhat P. D. *et al.*, (2007)** had developed buccoadhesive bilayered tablet comprising of drug containing bioadhesive layer and drug free backing layer to release the drug for extended period of time with reduction in dosing frequency. Carboplo-934P and methocel K4M in the ratio of 1:1 could be used to design effective and stable buccoadhesive Film of Terbutaline Sulphate.

15) **Nakhat P. D. *et al.*, (2007)** had formulated buccoadhesive tablet of Promethazine Hydrochloride to circumvent the first pass effect and to improve

its oral bioavailability with reduction in dosing frequency. All the formulations followed non- Fickian release mechanism. The optimized formulation F12 showed stability for the span of 6 months at 40±2° and 75±5% RH.

16) **Pramodkumar T. M. and Shivakumar H.G.**, **(2006)** prepared Buccoadhesive core-in-cup (BCC) systems of Terbutaline Sulphate by the direct compression method with polymers, like carbopols and hydroxy propyl methyl cellulose 4KM (HPMC 4KM) in ratios of 1: 0, 1: 1, 1: 2 and 0: 1. Buccoadhesive films were prepared by solvent evaporation using chitosan, HPMCK4M and HPMCP. Buccoadhesive core-in-cup systems and films of terbutaline sulphate can be developed as potential controlled release formulations for the treatment of bronchial asthma.

17) **Peppas N. A. *et al.*, (2006)** had developed novel acrylic based polymers that can be used as a mucoadhesive delivery system. The effects of different PEG-tethered structures on Residencewere studied using a tensionetric testing and the work of adhesion was calculated.

18) **Prachiti P. V. *et al.*, (2006)** provides an overview of buccal drug delivery system which includes various dosage forms like patch, film, film, microspheres and their evaluation tests and also given brief review of patents on buccal drug delivery system.

19) **Cafaaggi S. *et al.*, (2005)** had prepared and evaluated a matrix for buccal drug delivery composed of a Chitosan salt and poloxamer 407. The matrix composed of chitosan lactate and poloxamer 407 showed the best characteristics for buccal administration.

20) **Satyabratha B. *et al.,* (2005)** designed and evaluated the controlled release of mucoadhesive buccal tablet of Captopril with a goal to increase the bioavailability, reduced dosing frequency and improve patient compliance. The results indicate that the mucoadhesive buccal Film of Captopril may be a good choice to bypass the extensive hepatic first pass metabolism with an improvement in the bioavailability.

21) **Johnston T. P. *et al.,* (2005)** highlighted the use of mucoadhesive polymers in buccal drug delivery. Starting with a review of the oral mucosa, mechanism of drug permeation, and characteristics of desired polymers, it also covered the theories behind the adhesion of bioadhesive polymers to the mucosal epithelium.

22) **Ayyappan T. and Kasture P.V., (2005)** had developed and evaluated a buccoadhesive Ondansetron Hydrochloride tablet formulation using various mucoadhesive polymers in varying ratios. From this study, they concluded that tablet prepared from Carbopol 934P and Sodium carboxy methyl cellulose in a ratio of 1:4 exhibited the maximum drug release in 8 hr as compared to other polymeric ratios.

23) **Akbari J. *et al.,* (2004)** reported the effects of fillers on the release of Propranolol Hydrochloride. The result indicated that the presence of fillers increases dissolution rate of the drug**.** The release data also showed that the effect of lactose on the dissolution rate was greater than the DCP.

24) **Park C. and Munday D. L., (2002)** prepared and evaluated to determine the suitability of the formulation as a nicotine replacement product to aid in

smoking cessation. A combination of 20% w/w Carbopol 934 and 20% w/w HPC was thus found to provide suitable adhesion and controlled drug release.

25) **Johnston T. P. *et al.,* (1999)** had evaluated the gum from Hakea Gibbosa as a sustained release and mucoadhesive component in buccal Film following their application to the buccal mucosa of rabbits. The mucoadhesive buccal Film evaluated represent an improved transbuccal delivery system for conventional drug substances.

26) **Javed A. *et al.,* (1999)** prepared the buccoadhesive carriers of Triamcinolone Acetomide using different bioadhesive polymers in the different ratio in order to study effect on drug release and bioadhesion. The formulation containing 8 mg of triamcinolone acetomide, 2 magnesium stearate along with carbopol-934P and sodium carboxy methyl cellulose in the ratio of 1:4 was found to release the drug for period of 8 hours without getting dislodged.

27) **Shojaei A. H., (1998)** described that, within the oral mucosal cavity, the buccal region offers an attractive route of administration for systemic drug delivery. The mucosa has a rich blood supply and it is relatively permeable. It is the objective of this article to review buccal drug delivery by discussing the structure and environment of the oral mucosa and the experimental methods used in assessing buccal drug permeation/absorption. Buccal dosage forms will also be reviewed with an emphasis on bioadhesive polymeric based delivery systems.

28) **Agarwal S. P. *et al.,* (1996)** had prepared buccoadhesive erodible Film for local delivery of Clotrimazole to the oral cavity were developed using different bioadhesive polymers along with suitable excipients. The in vitro adhesion time

and release characteristics were found to be function of the type of polymer and also the total composition of the Film.

29) **Hung S. C. and Ahmad M. M., (1995)** developed a new and simple dissolution apparatus which is capable of evaluating the release of drug and bioadhesive properties of buccal Film. Film with higher concentrations of HPMC provide more prolonged release of drug. However they can be dislodging from the membrane more easily.

30) **Suraj P. A. *et al*., (1995)** had developed mucoadhesive buccal drug delivery system, multilayered Film of Diltiazem Hydrochloride were prepared which gave an in vitro drug release of 86.00%. In-situ testing was done using bovine cheek pouch membrane in a Franz diffusion cell.

DRUG

AND

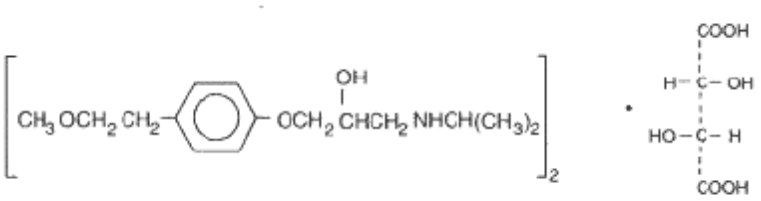
POLYMER PROFILE

2.2. DRUG PROFILE:- ***(****http:// www.google.com, drug bank; http://www.rxlist.;*

Anthony C. *et al., 2004; Tripathi K.D., 2004; PoptaniSanjay D .et al.,2012)*

METOPROLOL TARTRATE:

Chemical structure:



Chemical name : (±)-1-(Isopropylamino)-3-[p-(2-methoxyethyl)phenoxy]-2-

Propanol L-(+)-tartrate

Molecular Weight: 684.8

Molecular Formula: $(C_{15}H_{25}NO_3)_2\ C_4H_6O_6$

Category:

Anti adrenergic agents, adrenergic beta-Antagonists, Anti-Arrhythmia Agents, Antihypertensive Agents, and Sympatholytics.

Dose:

Conventional dose:- Initially 50 to 100 mg daily in a single or divided doses may increases weekly to 400 mg daily.

Maintenance dose:- 100 to 200 mg daily

Extended release preparation:- 25 to 100 mg once daily.

Description: Metoprolol tartrate USP is a white, practically odorless.

Solubility:

It is very soluble in water and 0.1 N HCl freely soluble in methylene chloride, in chloroform,and in alcohol; slightly soluble in acetone; and insoluble in ether.

Melting Point: 120 to 122° C (Succinate 136-138 °C)

Storage: Preserve in well-closed containers, at temperature not exceeding 25^{o} C.

Mechanism of action:

Metoprolol competes with adrenergic neurotransmitters such as catecholamines for binding at beta(1)-adrenergic receptors in the heart and vascular smooth muscle. Beta(1)-receptor blockade results in a decrease in heart rate, cardiac output, blood pressure.

Pharmacology:

Metoprolol, a competitive, beta1-selective (cardioselective) adrenergic antagonist, is similar to atenolol in its moderate lipid solubility, lack of intrinsic sympathomimetic activity (ISA), and weak membrane stabilizing activity (MSA).

Pharmacokinetic profile:

Metoprolol tartrate is absorbed rapid and completely from upper part of GIT.

P_{Ka}	:	9.7.
Partition coefficient	:	1.9 (Octanol / 0.1 N HCl)
V_d	:	4L/ Kg.
Half-life	:	3 to 4 hrs.
Body Clearance	:	13 ml/min/kg.
Protein Binding	:	11%.
Oral Bioavailability	:	40 to 50 %.

Therapeutic uses:

- Hypertension ,
- Angina pectoris,
- Cardiac arrhythmias,
- Myocardial infraction,
- Migraine prophylaxis and Hyperthyroidism.

Drug interactions:

- Beta-blocker and Calcium channel blocker have additive effect on the cardiac conducting system.
- Catecholamine depleting drug like Reserpine have additive effect with beta-blocker.
- Phenytoin, rifampicin, and phenobarbital induce hepatic biotransformation of enzymes and may decrease plasma concentration of beta blockers, Cimetidine and hydralazine may increase the bioavailability of agents such as propranolol and metoprolol by affecting hepatic blood flow.

Contraindication:

Hypertension and Angina: Extended release metoprolol tartrate is contraindicated in sinus bradycardia, heart block greater than first degree, cardiogenic shock.

Toxicity:

LD_{50}=5500 mg/kg (orally in rats), toxic effects include bradycardia, hypotension, bronchospasm, and cardiac failure. LD_{50}=2090 mg/kg (orally in mice).

Dosage forms:

Table 2.1. Dosage forms and routes of administration of Metoprolol

Form	Route
Liquid	Intravenous
Solution	Intravenous
Film	Oral
Film extended release	Oral

Marketed preparations:

Beloc, Betaloc, Lopresor, Lopresoretic, Lopressor, Lopressor, HCT,Metroprolol,

Prelis, Selo-Zok, Seloken, Selopral, Toprol, Toprol- XL

2.3. POLYMERS PROFILES: - *(Raymond C. Rowe, 2003)*

2.3.1. CARBOMER (CARBOPOL)

1. Nonproprietary Names:

BP: Carbomers

PhEur: Carbomera

USPNF: Carbomer

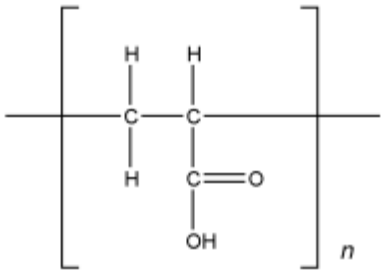
2. Synonyms:

Acritamer; acrylic acid polymer; Carbopol; carboxy polymethylene, polyacrylic acid; carboxyvinyl polymer; Pemulen; Ultrez.

3. Chemical Name and CAS Registry Number: Carbomer [9003-01-4]

4. Molecular Weight: 86,000

5. Structural Formula:



Acrylic acid monomer unit in carbomer resins.

Carbomer polymers are formed from repeating units of acrylic acid. The monomer unit is shown above. The polymer chains are crosslinked with allyl sucrose or allyl pentaerythritol.

6. Functional Category:

Bioadhesive; emulsifying agent; release-modifying agent; suspending agent; film binder; viscosity-increasing agent.

7. Applications in Pharmaceutical Formulation or Technology:

Carbomers are mainly used in liquid or semisolid pharmaceutical formulations as suspending or viscosity-increasing agents. Formulations include creams, gels, and ointments for use in ophthalmic, rectal, and topical preparations. Carbomer grades, even with a low residual benzene content, such as carbomer 934P, are no longer included in the PhEur 2005. Carbomer having low residuals only of ethyl acetate, such as carbomer 971P or 974P, may be used in oral preparations, in suspensions, Film, or sustained release film formulations.In film formulations, carbomers are used as dry or wet binders and as a rate controlling excipient. In wet granulation processes, water or an alcohol–water blend is used as the granulating fluid. Anhydrous organic solvents have also been used, with the inclusion of a polymeric binder. The tackiness of the wet mass can be reduced with the addition of certain cationic species to the granulating fluid or, in the case of water, with talc in the formulation.

Uses	Concentrations (%)
Emulsifying agent	0.1–0.5
Gelling agent	0.5–2.0
Suspending agent	0.5–1.0
Film binder	5.0–10.0

8. Description:

Carbomers are white-colored, 'fluffy', acidic, hygroscopic powders with a slight characteristic odor.

9. Typical Properties:-

Acidity/alkalinity : pH = 2.7–3.5 for a 0.5% w/v aqueous dispersion;

pH = 2.5–3.0 for a 1% w/v aqueous dispersion.

Density (bulk) : 1.76–2.08 g/cm3

Density (tapped) : 1.4 g/cm3

Melting point : Decomposition occurs within 50 minutes at 260°C.

Moisture content:

Normal water content is up to 2% w/w. However, carbomers are hygroscopic and typical equilibrium moisture content at 25°C and 50% relative humidity is 8–10% w/w. The moisture content of a carbomer does not affect its thickening efficiency, but an increase in the moisture content makes the carbomer more difficult to handle because it is less readily dispersed.

Solubility:

Soluble in water and, after neutralization, in ethanol (95%) and glycerin. Although they are described as 'soluble', carbomers do not dissolve but merely swell to a remarkable extent, since they are three-dimensionally crosslinked microgels. Furthermore, the pharmacopeial specifications are unclear, in that neutralization with

long-chain aliphatic amines or ethoxylated long-chain amines is required for swellability in ethanol, and with water-soluble amines for swellability in glycerin.

Viscosity (dynamic):

Carbomers disperse in water to form acidic colloidal dispersions of low viscosity that, when neutralized, produce highly viscous gels. Carbomer powders should first be dispersed into vigorously stirred water, taking care to avoid the formation of indispersible lumps, thenneutralized by the addition of a base. The Carbopol ETD and Ultrez 10 series of carbomerswas introduced to overcome some of the problems of dispersing the powder into aqueous solvents. These carbomer resins wet quickly yet hydrate slowly, while possessing a lower unneutralized dispersion viscosity.

10. Stability and Storage Conditions:

Carbomers are stable, hygroscopic materials that may be heated at temperatures below 104°C for up to 2 hours without affecting their thickening efficiency. However, exposure to excessive temperatures can result in discoloration and reduced stability.

2.3.2. HYPROMELLOSE (HYDROXYPROPYL METHYLCELLULOSE)

1. Nonproprietary Names:

BP: Hypromellose

JP: Hydroxypropylmethylcellulose

PhEur: Hypromellosum

USP: Hypromellose

2. Synonyms:

Benecel MHPC; E464; hydroxypropyl methylcellulose; HPMC; Methocel; methylcellulose propylene glycol ether; methyl hydroxypropylcellulose; Metolose; Tylopur.

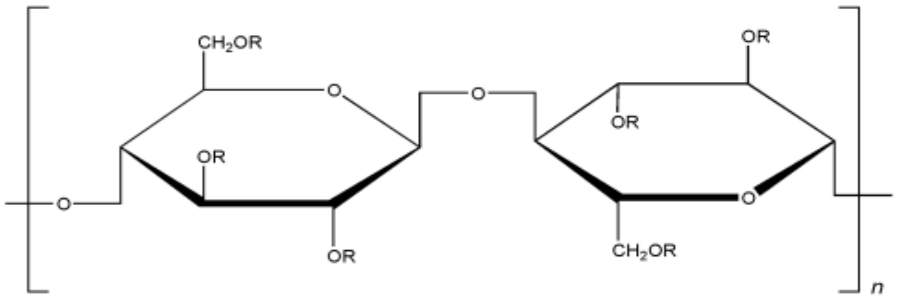
3. Chemical Name and CAS Registry Number:

Cellulose hydroxypropyl methyl ether [9004-65-3]

4. Molecular Weight:

Molecular weight is approximately 10 000–1 500 000.

5. Structural Formula:



Where R is H, CH_3, or CH_3CH (OH) CH_2

6. Functional Category:

Coating agent; film-former; rate-controlling polymer for sustained release; stabilizing agent;suspending agent; film binder; viscosity-increasing agent.

7. Applications in Pharmaceutical Formulation or Technology:

In oral products, hypromellose is primarily used as a film binder, in film-coating, and as matrix for use in extended-release film formulations. Concentrations between 2% and 5% w/w may be used as a binder in either wet- or dry-granulation processes. High-viscosity grades may be used to retard the release of drugs from a matrix at levels of 10–80% w/w in Film and capsules. Depending upon the viscosity grade, concentrations of 2–20% w/w are used for film-forming solutions to film-coat Film. Hypromellose at concentrations between 0.45–1.0% w/w may be added as a thickening agent to vehicles for eye drops and artificial tear solutions.

8. Description:

Hypromellose is an odorless and tasteless, white or creamy-white fibrous or granular powder.

9. Typical Properties:-

Acidity/alkalinity **:** pH = 5.5–8.0 for a 1% w/w aqueous solution.

Ash **:** 1.5–3.0%, depending upon the grade and viscosity.

Autoignition temperature **:** 360°C

Density (bulk) **:** 0.341 g/cm3

Density (tapped) **:** 0.557 g/cm3

Density (true) : 1.326 g/cm3

Melting point : Browns at 190–200°C; chars at 225–250°C.

Glass transition temperature is 170–180°C.

Moisture content:

Hypromellose absorbs moisture from the atmosphere; the amount of water absorbed depends upon the initial moisture content and the temperature and relative humidity of the surrounding air.

Solubility:

Soluble in cold water, forming a viscous colloidal solution; practically insoluble in chloroform, ethanol (95%), and ether, but soluble in mixtures of ethanol and dichloromethane, mixtures of methanol and dichloromethane, and mixtures of water and alcohol. Certain grades of hypromellose are soluble in aqueous acetone solutions, mixtures of dichloromethane and propan-2-ol, and other organic solvents.

Viscosity (dynamic):

A wide range of viscosity types are commercially available. Aqueous solutions are most commonly prepared, although hypromellose may also be dissolved in aqueous alcohols such as ethanol and propane-2-ol provided the alcohol content is less than 50% w/w.

Typical viscosity values for 2% (w/v) aqueous solutions of Methocel (Dow Chemical Co.). Viscosities measured at 20°C.

10. Stability and Storage Conditions:

Hypromellose powder is a stable material, although it is hygroscopic after drying. Solutions are stable at pH 3–11. Increasing temperature reduces the viscosity of solutions. Hypromellose undergoes a reversible sol–gel transformation upon heating and cooling, respectively. The gel point is 50–90°C, depending upon the grade and concentration of material.

11. Incompatibilities:

Hypromellose is incompatible with some oxidizing agents. Since it is nonionic, hypromellose will not complex with metallic salts or ionic organics to form insoluble precipitates.

AIM

AND

OBJECTIVES

3. AIM AND OBJECTIVES

Metoprolol tartrate is a selective β-1 adrenergic antagonist used in the treatment of the cardiovascular system, especially Hypertension. This drug, with 12% oral bioavailability and having half-life of 3 to 4 hours, is readily and completely absorbed from the gastro intestinal tract but is subjected to considerable first-pass metabolism. It is a "class-I" drug according to Biopharmaceutics classification system (BCS), possessing both high solubility and high permeability absorption characteristics. It has a short elimination half-life and rapidly absorbed in gastrointestinal tract. If it is formulated by conventional tablets requires multiple daily administration (3-4 times daily) with resulting in convenience to the patient and the possibility of reduced compliance with prescribed therapy.

These physico-chemical properties of metoprolol tartrate make its suitable candidate for administration by buccal route.

One of the significant approaches in the modern drug delivery systems is to target the drug in particular part of the body. Mucosal surface has attracted attention of scientific community in the living body; mucosal surfaces are available in the oral cavity especially to the buccal region.

The present study focused on the delivery of drug via buccal mucosa. Drug or the dosage forms have to exhibit mucoadhesive properties to remain static at the site of application. Oral mucosa is robust and shows short recovery time after stress or damage. Mucoadhesive dosage forms are readily localized in the region and prolonged resistance time and absorption of the drug at the site of administration.

These dosage forms facilitate intimate contact of the formulation with the underlying absorption surface.

Buccal drug delivery has been considered as an alternative to oral dosing for compound subjected to degradation in the GIT or to undergo extensive first pass metabolism. Buccal drug delivery offers a safer mode of utilization, since drug absorption can be promptly terminated in case of toxicity by removing the dosage form from the buccal cavity.

An attempt was taken to develop the buccoadhesive Film with this drug to minimize the fluctuations in blood concentration by avoiding first pass effect, decreasing the risk of side effects and show uniform pharmacological response.

The aim of present work was to formulate and evaluate buccoadhesive Film of Metoprolol tartrate containing 50 mg of drug, using a mucoadhesive polymer with the help of solvent casting method in order to the release for the period of 8 hours.

This type of formulation will ensure minimum fluctuations in the plasma drug concentration and reduced dosing frequency which in turn will result into improved patient compliance.

PLAN

OF

WORK

4. PLAN OF WORK

- **LITERATURE SURVEY**
- **SELECTION OF DRUG AND POLYMERS**
- **PROCUREMENT OF DRUG AND POLYMERS**
- **EXPERIMENTAL WORK**
  - ✓ **Preformulation Study**

Identification of Drug

- Organoleptic Properties
- Determination of Melting Point
- Solubility Study
- FTIR
- UV Spectrophotometric Study
- Assay of Metoprolol tartrate
- Differential Scanning Calorimetry (DSC)

✓ **Formulation Design of 2^3 full factorial design**

✓ **Formulation of Buccoadhesive film**

1. **Backing layer**
2. **Buccoadhesive layer containing drug**

✓ **Evaluation of Buccoadhesive film**

- Appearance
- Film thickness & determination of weight of film
- Folding endurance
- Swelling index
- Surface pH
- Content uniformity
- *Ex Vivo* Buccoadhesive Strength
- *Ex Vivo* Residence Time

- *In Vitro* Drug release Study
- In Vitro Buccal permeation study
- Histopathological studies
- Kinetics modeling of Drug Release Profile
- Statistical Analysis of response by Design Expert software
- Stability Study

- **RESULTS AND DISCUSSION**
- **SUMMARY AND CONCLUSION**
- **FUTURE PROSPECTS**
- **BIBLIOGRAPHY**

MATERIALS AND EQUIPMENTS

5. MATERIALS AND EQUIPMENTS

5.1.MATERIALS USED

Table 5.1: List of Drug and Polymers with source

Sr.No.	Ingredients	Supplier
1	Metoprolol tartrate	Madras Pharmaceuticals Pvt Ltd,Chennai.
2	Carbopol-934P	Loba Chemie, Mumbai.
3	Hydroxy Propyl Methyl Cellulose K4M	Loba Chemie, Mumbai.
4	Di Methyl Sulphoxide	Loba Chemie, Mumbai.
5	Ethyl Cellulose	Loba Chemie, Mumbai.
6	Di Butyl Pthalate	Loba Chemie, Mumbai.
7	Ethanol	Loba Chemie, Mumbai.
8	Propylene Glycol	Loba Chemie, Mumbai.
9	Potassium di Hydrogen phosphate	Loba Chemie, Mumbai.
10	Sodium hydroxide	Loba Chemie, Mumbai.

5.2. EQUIPMENTS USED:

Table 5.2: List of Equipments with model/make

Sl. No.	Name of the Instruments	Make	Model
1	Electronic Balance	Shimadzu, Japan	BL- 200H.
2	UV-Visible Spectrophotometer	Shimadzu, Japan	1700
3	FTIR Spectrophotometer	Perkin elmer-Pharmaspec-1	----
4	USP, Type II Dissolution Test Apparatus	Veego scientifics, Mumbai	VDA – 8DR.
5	Digital pH Meter	Elico scientifics, Mumbai	L1610
6	Hot air oven	Prescision scientific co., Chennai	P-1401
7	Vernier Calipers	Indolabs, Chennai	---
8	Humidity Chamber	Labtech, Ambala	----
9	Melting Point Test Apparatus	Prescision scientific co., Chennai	----
10	Physical Balance	Fabricated assembly in lab	----
11	Differential Scanning Calorimeter	Shimadzu Japan	Q20V24.4
12	Magnetic Stirrer	Labtech, Ambala	----

PRE-FORMULATION STUDY

6. PRE-FORMULATION STUDY

6.1. Identification of Drug:- *(Anthony C. et al., 2004; Indian Pharmacopoeia, 2007)*

6.1.1. Organoleptic Properties:

The colour, odour and taste of the drug were recorded using descriptive terminology.

6.1.2. Melting Point:

Melting point of the drug was determined by capillary tube method.

6.1.3. Solubility Study:

It is important to know about solubility characteristic of a drug in aqueous system. Since they must possess some limited aqueous solubility to elicit a therapeutic response. The solubility of drug was recorded by using various descriptive terminology specified in Indian Pharmacopoeia, 2007.

6.1.4. UV Spectrophotometric Study:

The absorption maximum of the standard solution was scanned between 200-400 nm regions on UV-Visible Spectrophotometer. The absorption maximum obtained with the substance being examined corresponds in position and relative intensity to those in the reference spectrum.

Preparation of Standard Curve of Metoprolol tartrate:

- **Preparation of Solutions:**

Preparation of Phosphate Buffer pH 6.8: Phosphate buffers pH 6.8 was prepared according to I.P. A quantity of 50.0 ml of 0.2 M potassium dihydrogen phosphate in a 200 ml volumetric flask and add 22.4 ml of 0.2 M sodium hydroxide and then add water to volume.

Stock solution of Metoprolol tartrate was prepared by phosphate buffer pH 6.8. Accurately weighed 100 mg of Metoprolol tartrate was dissolved in little quantity of phosphate buffer pH 6.8 and volume was adjusted to 100 ml with the same to prepare standard solution having concentration of 100 µg/ ml.

- **Procedure:**

From the stock solution, aliquots of 1, 2,3,4,5 and 6 ml were transferred to 10 ml volumetric flasks and final volume was made to 10 ml with phosphate buffer pH 6.8. Absorbance values of these solutions were measured against blank at 274.5 nm using UV-Visible Spectrophotometer.

6.1.5. Percentage Purity of Drug:

Accurately weighed 10 mg of Metoprolol tartrate was dissolved in little quantity of phosphate buffer pH 6.8 and volume was adjusted to 100 ml with the same to prepare standard solution having concentration of 100 µg/ ml. From the above solution, aliquots of 5 ml were transferred to 10 ml volumetric flasks and final volume was made to 10 ml with phosphate buffer pH 6.8. Absorbance values of these solutions were measured against blank at 274.5 nm using UV-Visible

Spectrophotometer. The percentage purity of drug was calculated by using calibration graph method.

6.1.6. FTIR Study:-

Fourier Transforms Infra-Red (FTIR) Spectroscopy:

(Robert M. Silverstein, 2003; Becket A. H. and Stenlake J. B., 2005)

FTIR study was carried out to check identity of drug. Infrared spectrum of Metoprolol tartrate was determined on Fourier transform Infrared Spectrophotometer using KBr dispersion method. The base line correction was done using dried potassium bromide. Then the spectrum of dried mixture of drug and potassium bromide was run followed by drug by using FTIR spectrophotometer. The absorption maximums in spectrum obtained with the substance being examined correspond in position and relative intensity to those in the reference spectrum.

6.1.7. Drug – Polymers Compatibility Study by DSC Analysis:-

Determination of drug-polymer compatibility

The proper design and formulation of a dosage form requires consideration of the physical, chemical and biological characteristics of all drug substances and excipients to be used in the fabricating the product. Each polymer used in the formulations was blended with the drug levels that are realistic with respect to the final dosage form. Each polymer was thoroughly blended with drug to increase drug-polymer molecular contacts to accelerate the reactions if possible.

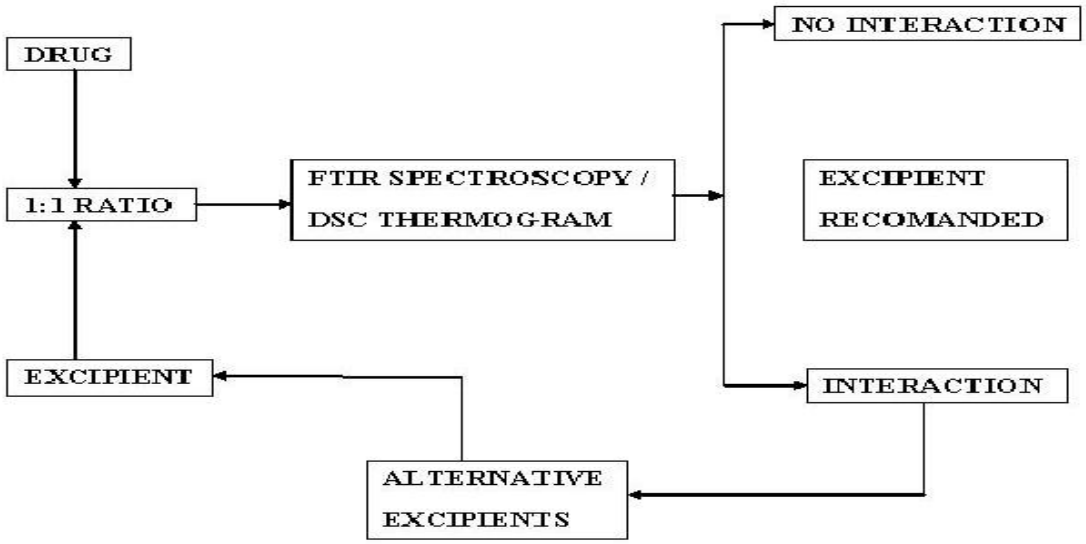


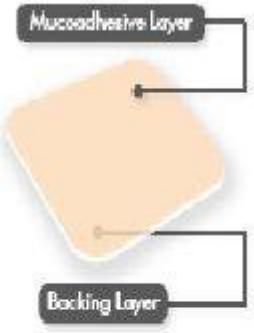
Figure 6.1: Schematic representation of compatibility studies

Differential scanning calorimetry (DSC):

(Chatwal G. R. and Anand S. K., 2007)

Any possible drug polymer interaction can be studied by thermal analysis. The DSC study was performed on pure drug, drug+ Carbopol 934 P and drug + HPMC K4M. The study was carried out using a Shimadzu DSC Q20 V24.4,116, (Japan). The 5 mg of sample were heated in a hermetically sealed aluminum pans in the temperature range of 25-500ºC at heating rate of 10ºC /min under nitrogen flow of 50ml/min.

FORMULATION OF BUCCOADHESIVE FILM



7. FORMULATION OF BUCCOADHESIVE FILM

Formulation design of 2 ^{3}full factorial design

A 2^3 randomized full factorial design was used in this study. Three factors were evaluated,each at two levels and experimental trials were performed on all eight possible combinations (Table 7.1). The amount of HPMC K4M as film former (X1), and the amount of carbopol 934P as buccoadhesive polymer (X2) and concentration of DMSO as penetration enhancer (X3) were selected as independent variables. The percent cumulative drug release (% CDR) at 8^{th} hour, *ex-vivo* residence time and cumulative % permeation at 8^{th} hour respectively were selected as dependent variables. Regression polynomials for the individual dependant variables were calculated with the help of Design Expert 8.0.2 software (Stat-Ease,Inc,USA) and applied to approximate the response surface and contour plots. The general model as shown below was generated-

$$Y=B0+B1X1+B2X2+B3X3+….+B12X1X2+B13X1X3+B23X2X3+….+B123X1X2X3$$

B1 is estimated coefficient for the factor X1,similarly B2 and B3 are estimated coefficients for the factor X2 and X3 respectively. The main effects (X1,X2 and X3) represent the average result of changing one factor at a time from its low to high value. The interaction terms show how the response changes when three factors are simultaneously changed.

Table 7.1: Composition of Bucccoadhesive Film of Metoprolol tartrate

Ingredients	**BF1**	**BF2**	**BF3**	**BF4**	**BF5**	**BF6**	**BF7**	**BF8**	**BF9**
Metoprolol tartrate (mg)	50	50	50	50	50	50	50	50	50
HPMC K4M %w/v (X1)	-1	-1	-1	-1	+1	+1	+1	+1	0
Carbopol 934P %w/v (X2)	-1	-1	+1	+1	-1	-1	+1	+1	0
DMSO %w/v (X3)	-1	+1	-1	+1	-1	+1	-1	+1	0
Independent variables values									
	+1			-1			0		
HPMCK4M (X1)	600 mg			300 mg			450 mg		
Carbopol 934P (X2)	100 mg			50 mg			75 mg		
DMSO (X3)	0.6 ml			0.3 ml			0.45 ml		

7.2. PREPARATION OF BUCCOADHESSIVE FILM:-

The buccoadhesive Film were prepared by solvent casting method. Each 2 cm film contained 50 mg of Metoprolol tartrate.

Backing layer:

For preparation of backing layer a glass petridish of 9.5 cm diameter was used as a casting surface. Backing membrane of ethyl cellulose was fabricated by slowly pouring a solution containing 500 mg of ethyl cellulose and 2 % dibutyl phthalate in 10 ml of ethanol to the glass petridish and air drying for 1 hr.

Buccoadhesive layer containing drug:

3% w/v HPMC K4M was dissolved in 10 ml of ethanol and water (3:2) under constant stirring till a clear solution was obtained. To this 1 % w/v neutralized carbopol 934P (0.5 g of carbopol 934P was neutralized by approximately 0.2 g of sodium hydroxide) and 5 % v/v propylene glycol was added with stirring using magnetic stirrer. Then sufficient amount of metoprolol tratrate was added with stirring so as to have 50 mg of drug per 2 cm diameter of film. The mixture was stored at low temperature in order to remove air bubbles. The resultant clear solution was then poured on performed backing layer of ethyl cellulose and allowed to dry undisturbed for 4 h at 60 ^{0}C in the oven to ensure complete removal of solvent. The dried film was cut into discs of 2 cm diameter and packed in aluminium foil and stored in desiccators.

7.3. CALCULATION OF DOSE FOR CONVERTING METOPROLOL SUCCINATE IN TO METOPROLOL TARTRATE:

Metoprolol succinate molecular weight =23.75

Metoprolol tartrate molecular weight=25.00

Conversion factor =1.052631579

50mg of metoprolol tartrate was equivalent to 52.63mg of metoprolol succinate.

7.4. CALCULATION OF DOSE FOR BUCCOADHESIVE FILMS:

2 cm of the buccoadhesive film contains 50 mg.

Diameter of petridish =9.5 cm

Calculation of dose:

9.5 cm of petridish contains drug=x

=(9.5/2)X50

= 237.5 mg

9.5 cm of petridish contains 237.5 mg of metoprolol tartrate.

EVALUATION OF BUCCOADHESIVE FILM

8. EVALUATION OF BUCCOADHESIVE FILM

8.1. Physical Properties of Film:-

(Lachmann L. Et al., 1987; Bankar G.S. and Rhodes C.T., 1996)

8.1.1. Appearance:

The formulated films visually observed for colour, clarity and transparency.

8.1.2. Dimension (Diameter and Thickness):

The Thickness and diameter permits accurate measurements and provide information on the variation between Films. The thickness and diameter of the Film was determined using a Vernier caliper. Three Films from each type of formulation were used and average values were calculated.

8.2. Folding endurance

(Pankajkumar, et al., 2012; Ravikumar Reddy, et al., 2012)

Folding Endurance of the film was determined by repeatedly folding the films at the same place till it breaks. The films was folded in the center, between finger and thumb and then opened. This was one folding. The number of times, the film could be folded at the same place without breaking gave the value of folding endurance.

8.3. Swelling studies

Weight method – swelling studies *(Pankajkumar, et al., 2012)*

The films were weighed individually (designated as Wo) and placed separately in 2% agar gel plates, incubated at $37+1^0$C and examined for any physical changes. At

regular 1hr time interval until 3 hours, films were removed from the gel plates and excess surface water was removed carefully using filter paper. The swollen films were then reweighed (W_T) and the swelling index were calculated using the following formula:

$$\% SW = [(W_T - W_O) / W_O] \times 100$$

Where,

%SW = percentage swelling index;

W_T = weight of swollen film after time T;

W_O = original weight of film at zero time;

8.4. Surface pH *(Ayyappan T. and Kasture P.V., 2005)*

Surface pH of oral cavity was determined in order to investigate the possibility of any side effects in a buccal mucosa. Attempt was made to keep the surface close to the saliva pH. The formulations were first wetted by adding 1ml distilled water to its surface. The surface pH was then recorded by bringing a glass electrode near the surface of the formulation and allowing it to equilibrate for 1min.

8.5. Drug content (Venkatalakshmi *et al*.,2011)

Uniformity of drug content was determined by assaying the individual films. Three films from each batch were powdered individually and each was dissolved in 100 mL of isotonic phosphate buffer pH 6.8 by stirring on a magnetic stirrer for 1 hours. The absorbance of each of these solutions was then measured on UV-visible spectrophotometer at 274.5 nm.

8.6. *Ex-vivo* Bioadhesion Study:-

a) Fabrication of the Test Assembly: *(Gupta A. et al., 1992)*

For *in-vitro* study, an apparatus designed for the determination of mucoadhesive bond force was used. Bioadhesion test assembly is shown in figure 8.1.

For the designing of the apparatus, two pan weighing balance was used. The pan from the left side was replaced with a glass vial hanged with the thread. Another glass vial inside the glass bottle was placed below this vial in such a way that both (upper and lower) vials just touch each other. The two sides were balanced so that the right side exactly 2 gm heavier than left side by placing appropriate weight in right side pans.

Using this bioadhesion test assembly, the bioadhesion strength expressed in weight (g) required for the detachment of the film from the mucosa was determined.

b) Measurement of Adhesion Force: *(Cafaggi S. et al., 2005)*

Measurement of adhesion force was determined by using bovine buccal mucosa which was obtained from slaughter house. The underlying tissues were separated and washed thoroughly with phosphate buffer solution (pH 6.8). The membrane was then tied to the bottom of the lower vial using rubber band. The vial was kept in glass bottle which was filled with phosphate buffer solution at 37 ± 1 ^{0}C in such way that buffer just reaches the surface of mucosal membrane and kept it moist. The films to be tested was stuck on the lower side of the hanging Glass vial by using adhesive tape and the weight (2 gm)

Fig. 8.1: Bioadhesion test assembly

on the right pan was removed. This lowered the left side of the pan along with the film over the mucosa. It was kept undisturbed for three minutes and the weights are added on right side of pan till the film just separated from the membrane surface. The excess weight on the right pan i.e. total weight minus 2 gm was taken as measure of bioadhesive strength. Bioadhesive force was calculated by using following equation.

$$\text{Bioadhesive force} = \frac{\text{Bioadhesive Strength}}{100} \times 9.81$$

8.7. *Ex-Vivo* Residence Time: - *(Patel V. M. et al., 2007)*

The *ex-vivo* Residence time was examined after application of the buccal film on freshly cut goat buccal mucosa. The fresh goat buccal mucosa was tied on the glass slide and a mucoadhesive core side of each film was wet with 1 drop of phosphate buffer (pH 6.8) and pasted to the goat buccal mucosa by applying a light force with a fingertip for 50 seconds. The glass slide was then put in the beaker, which was filled with 200 ml of the phosphate buffer and kept at 37°C± 1°C. After 2 minutes, a slow stirring rate was applied to stimulate the buccal cavity environment and film adhesion was monitored for 14 hours. The time for the film to detach from the goat buccal mucosa was recorded as the Residence time.

8.8. *In- Vitro* Drug Release Study:-

(Nagendra kumar et al.,2011)

The influence of technologically defined condition and difficulty in simulating *in- vivo* conditions has led to the development of a number of *in- vitro* release methods for buccal formulations, however, no standard method has yet been developed. *In-vitro* release rate of buccoadhesive Film of Metoprolol tartrate was carried out using rotating paddle apparatus (USP Type II). The dissolution medium consisted of 250 ml of phosphate buffer (pH 6.8). The release study was performed at 37°C ± 0.5°C with a rotation speed of 50 rpm. The sample (5 ml) was withdrawn at time interval of 30 and 60 minutes up to 8 h and replaced with 5 ml of dissolution media each time to maintain the sink conditions. The amount of Metoprolol tartrate released was determined spectrophotometrically at 274.5 nm.

Table 8.1: Parameters were used for the dissolution study

Apparatus	USP Dissolution apparatus (Type II)
Dissolution medium	Phosphate buffer (pH 6.8)
Temperature	37 ± 0.5 °C
Volume	250 ml
Speed	50 rpm
Sample withdrawn	5 ml
Running Time	8 hrs

8.9.*In-vitro* buccal permeation: (*Nagaraju.K et al., 2011)*

The *in-vitro* buccal permeation study of metoprolol tartrate through goat buccal mucosa was performed using Franz diffusion cell. A specimen of fresh goat buccal mucosa was mounted between donar and receptor compartments. The film was placed on the mucosa, and the compartments were filled with 1ml of phosphate buffer pH 6.8. The receptor compartment was filled with isotonic phosphate buffer pH 6.8 maintained at 37.0±0.2°C and hydrodynamics in the receptor compartment were maintained by stirring magnetically at 50 rpm. Aliquots of 1 ml sample were withdrawn at predetermined time intervals and analyzed UV spectrophotometer at 274.5nm.

Fig. 8.2: Franz diffusion assembly

8.10. Histopathological Studies:

Histopathological evaluation of goat buccal mucosa tissue (control)incubated in phosphate buffer saline solution pH 6.8 was compared with that treated with buccal film for 8 hr. the tissue was properly washed twice using normal saline solution to remove the adhered tissues and protein. The tissue was fixed with 10 %formalin, routinely processed and set in paraffin. Paraffin sections were cut on glass slides and stained with hematoxylin and eosin. Examine the transverse sections of treated goat buccal mucosa under light microscope to detect any cellular damage to buccal mucosa tissue.

8.11. Kinetics of *In-vitro* Drug Release: *(Brahmankar D.M. and Jaiswal S.B., 2006)*

To study the release kinetics of *in-vitro* drug release, data was applied to kinetic models such as zero order, first order, Higuchi and Korsmeyer- Peppas.

- **Zero order:**

$$C = K_0 t$$

Where K_0 - is the zero-order rate constant expressed in units of concentration/time

t -is the time in hrs.

- **First order:**

$$LogC = LogC_0 - Kt / 2.503$$

Where C_0 - is the initial concentration of drug,

K - is the first order constant

t - is the time in hrs.

- **Higuchi:**

$$Qt = Kt^{1/2}$$

Where Q_t - is the amount of the release drug in time t,

K- is the kinetic constant and

t- is time in hrs.

- **Korsmeyer Peppas:**

$$Mt / M\infty = Kt\ n$$

Where M_t - represents amount of the released drug at time t,

M_∞ - is the overall amount of the drug (whole dose) released after 8 hrs

K - is the diffusion characteristic of drug/ polymer system constant

n - is a diffusional exponent that characterizes the mechanism of release of drug.

The value of n indicates the drug release mechanism related to the geometrical shape of the delivery system, if the exponent n = 0.5, then the drug release mechanism is Fickian diffusion. If n < 0.5 the mechanism is quasi-Fickian diffusion, and 0.5 < n < 1.0, then it is non-Fickian or anomalous diffusion and when n = 1.0 mechanism is non Fickian case II diffusion, n> 1.0 mechanism is non Fickian super case II.

8.12. Statistical analysis of response by design expert software:

(Prakash Rao B.et al.,2011)

Design Expert 8.0.2 software was used for the analysis of effect of each variable on the designated response. Pareto charts were made for the analysis of each response coefficient for its statistical significance. Quantitative and qualitative contribution of each variable on each of the response was analyzed. The significant response polynomial equations generated by design expert were used to validate the statistical design. Response surface pictelots were generated to visualize the simultaneous effect of each variable on each response parameter. Possible interactions between X1X2, X2X3, and X1X3 were also studied and analyzed.

Validation of Experimental Design:

The polynomial equations were utilized for validation of the experimental design. An extra check point formulation BF9 was prepared with the predicted value for of *in-vitro* drug release (%CDR at 8^{th} hr),Cumulative permeability at 8^{th}hr and

ex-vivo residence time. Experimental value were determined by formulating and evaluating BF9 and close resemblance between predicted and experimental value indicated validity of the generated model .Finally an optimized formulation was selected on the basis of higher *in-vitro* drug after 8hr (%CDR),higher ex-vivo residence time ,and higher cumulative %permeability at 8%hr with good desirability factor using software analysis.

8.13. Stability Study: - *(Nakhat P. D. et al., 2007)*

The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light, enabling recommended storage conditions, re-test periods and shelf-lives. Generally, the observation of the rate at which the product degrades under normal room temperature requires a long time. To avoid this undesirable delay, the principles of accelerated stability studies are adopted.

From the prepared film formulation BF4 showed appropriate balance between *In-vitro* drug release and bioadhesive property, Hence formulation BF4 was selected for the stability study. The study was carried out to observe the effect of temperature on optimized formulation (BF4). Stability studies were carried out at 40° C / 75% RH for the formulation BF4 for 3 months. The buccal mucoadhesive Film were stored at 40°C/75% RH in closed high density polyethylene bottles for 3 months. The samples were withdrawn after periods of 1 month, 2 month and 3 month. The samples were analyzed for its Appearance, Surface pH , Ex-vivo residence time, Drug content and *In vitro* drug permeation.

RESULTS AND DISCUSSION

9. RESULTS AND DISCUSSION

9.1. Identification of Drug:-

9.1.1. Organoleptic Properties:

Colour : White

Odour : Odorless

Taste : Tasteless

Appearance: Fine powder

9.1.2. Melting Point:

Melting point values of sample utilized in the formulation was found to be in range of 136°C. Hence, results were complied the limits specified in official Book.

9.1.3. Solubility Study:

Table 9.1: The solubility of Metoprolol tartrate in various solvents

Name of solvent	Parts of solvent required per part of solute	Solubility
Distilled water	10	Very Soluble
Ethanol (95%)	40	Freely soluble
Chloroform	400	Sparingly soluble
Ether	600	Practically insoluble
Phosphate buffer pH 6.8	50	Freely soluble
Phosphate buffer pH 7.4	70	Freely soluble

9.1.4. UV Spectrophotometric Study:

The absorption maximum for Metoprolol tartrate was found to be 274.5 nm.

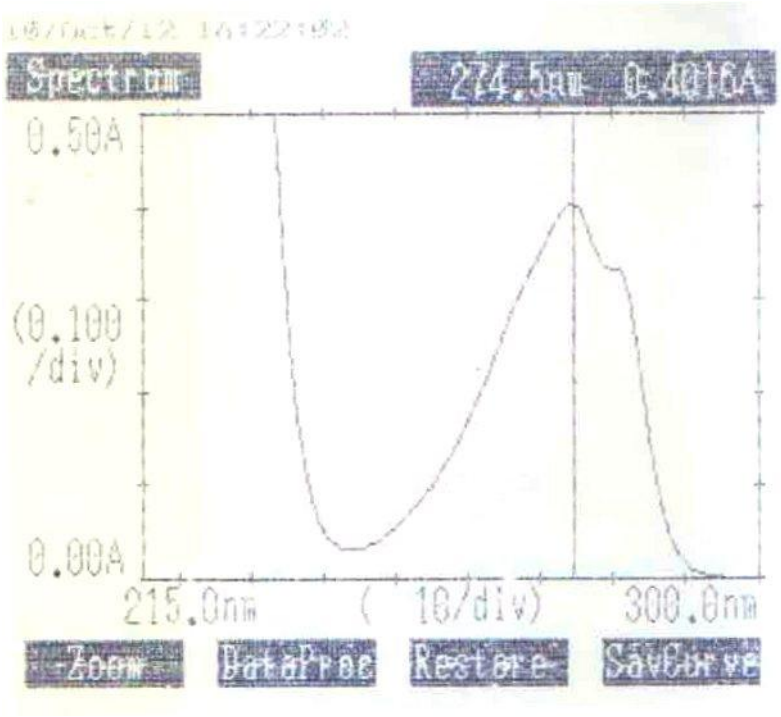


Fig. 9.1: λmax of Metoprolol tartrate in phosphate buffer pH 6.8

9.1.5. Calibration Curve of Metoprolol tartrate:

UV absorption spectrum of Metoprolol tartrate in phosphate buffer pH 6.8 shows λmax at 274.5 nm. Absorbances obtained for various concentrations of Metoprolol tartrate are given in Table 9.2. The graph of absorbance vs. concentration for Metoprolol tartrate was found to be linear in the concentration range of 10-60 µg /ml. This drug obeys Beer- Lambert's law in the range of 10-60 µg /ml.

Table 9.2: Data of concentration and absorbance for Metoprolol tartrate in Phosphate buffer pH 6.8

Sr. No.	Concentration (µg/ml)	Absorbance
1	0	0.000
2	10	0.040
3	20	0.081
4	30	0.120
5	40	0.165
6	50	0.201
7	60	0.242

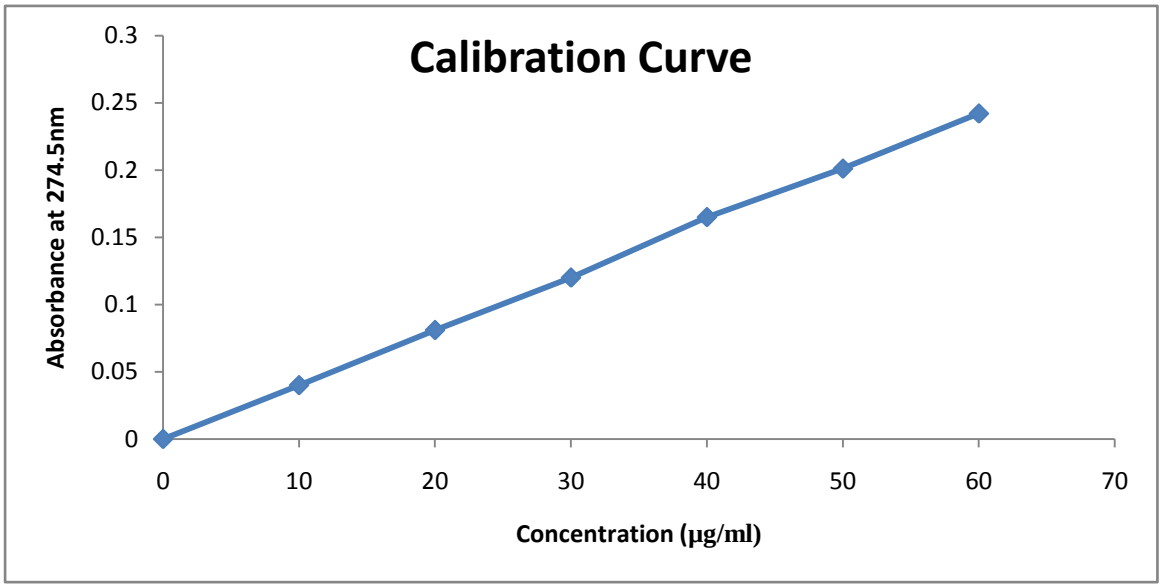


Fig. 9.2: Standard graph of Metoprolol tartrate in Phosphate buffer pH 6.8

Table 9.3: Data for Calibration Curve Parameters

Sr. No.	Parameters	Values
1	Correlation coefficient (r)	0.9998
2	Slope	0.247
3	Intercept	0.0094

9.1.6. Percentage Purity of Drug:

The percentage purity of drug was calculated by using calibration graph method.

Table 9.4: Percentage purity of drug

Sr. No.	Percentage purity (%)	Average percentage purity (%)
1	99.56	99.58
2	99.82	
3	99.38	

The official percentage purity of Metoprolol tartrate is not less than 99.00% and not more than 101.00%. So, it can be declared as pure drug.

9.1.7. Fourier Transforms Infra-Red (FTIR) Study:

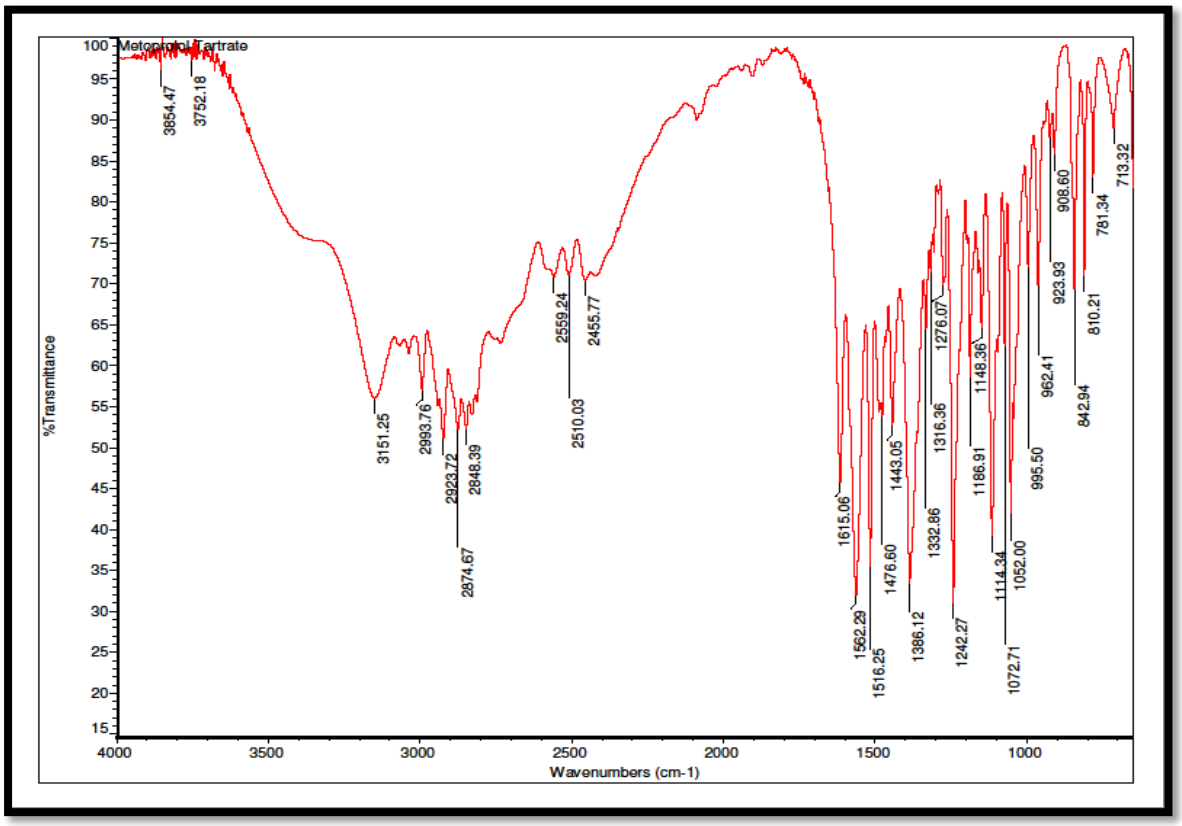


Fig. 9.3: FT-IR spectra of Metoprolol tartrate

Table 9.5: Interpretation of FTIR spectra of Metoprolol tartrate

O-H Stretching	3752.18
C-O-C Stretching Aliphatic	1114.34
C- N Stretching	1242.27
N-H bend Aliphatic	1615.06
N-H Stretch 2 Amine	3151.25

From the above figure 9.3, it can be seen that, the major functional group peaks observed in spectra of Drug with all the polymers remains unchanged as compared with spectra of Metoprolol tartrate. So from the above IR spectra it can be observed that the identified as Metoprolol tartrate.

9.2. Drug – polymer compatibility Study by DSC Analysis

Differential Scanning Calorimetry (DSC) Analysis:

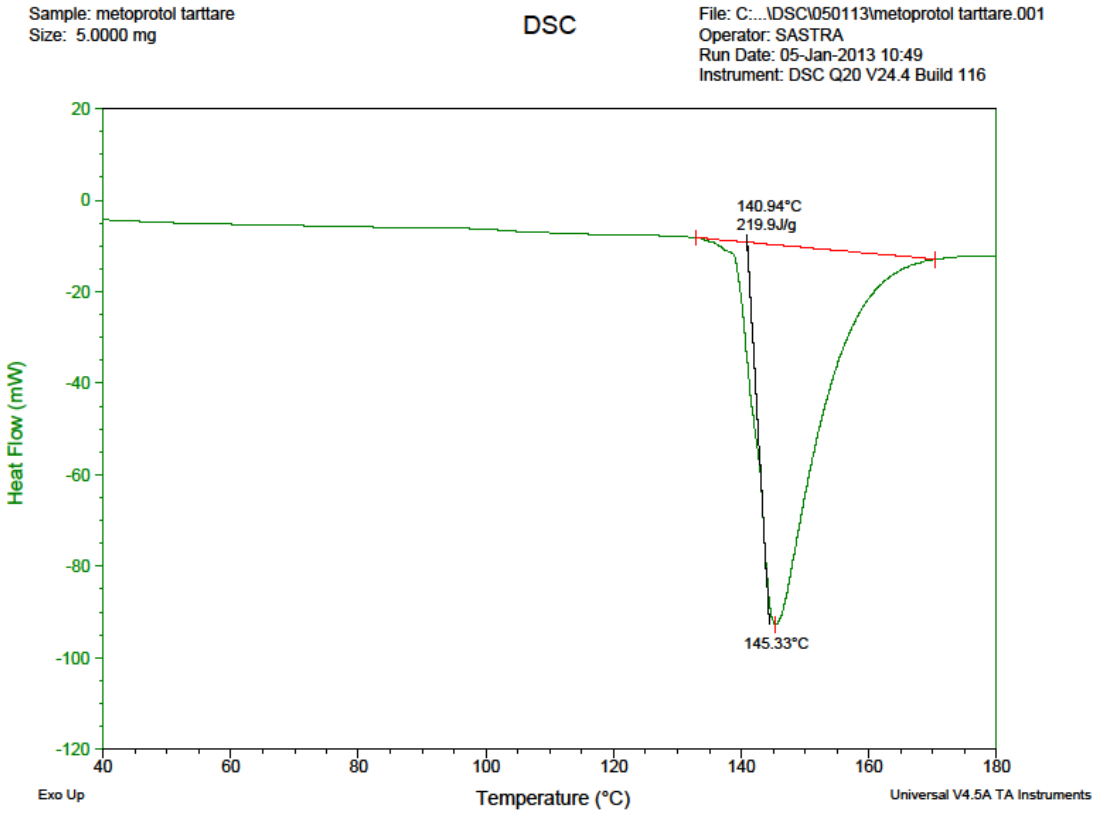


Fig. 9.4: DSC thermo gram of Metoprolol tartrate

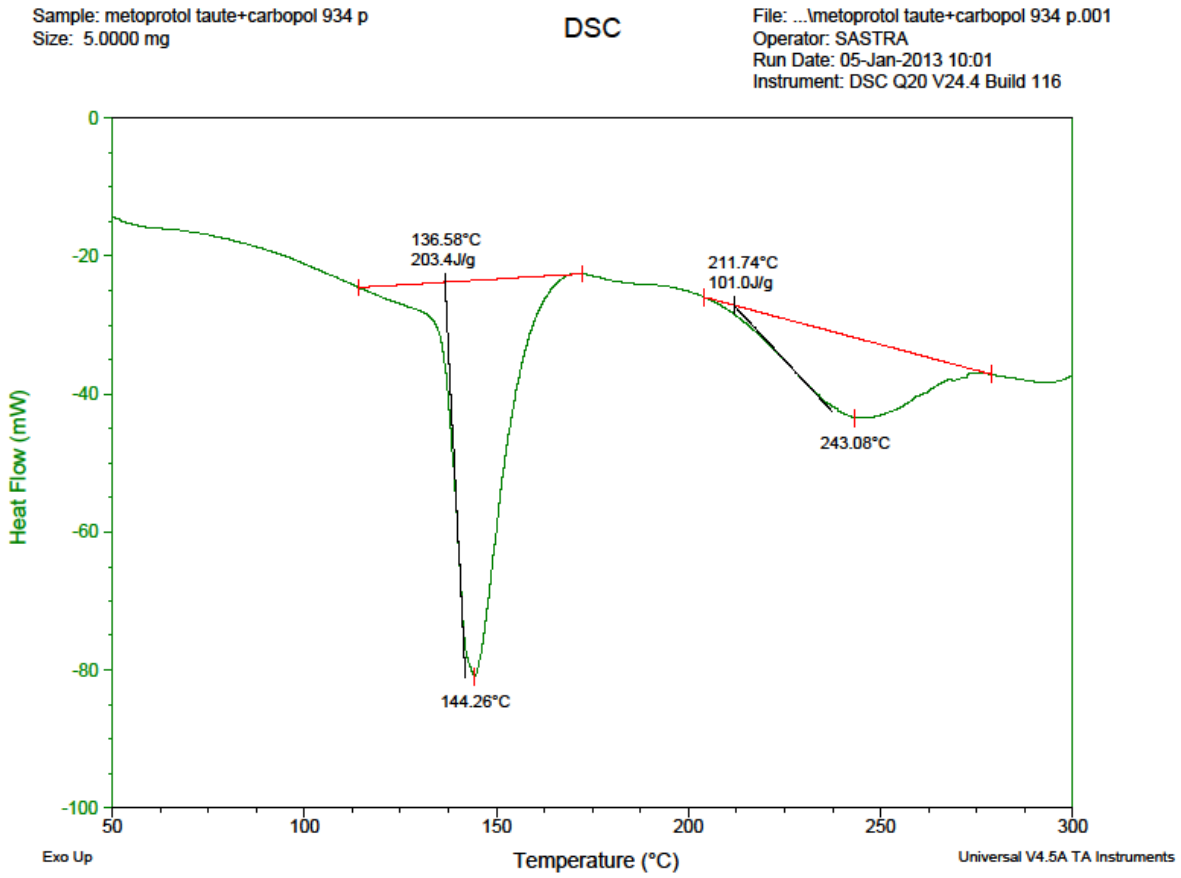


Fig. 9.5: DSC thermo gram of Metoprolol tartrate + Carbopol 934 P

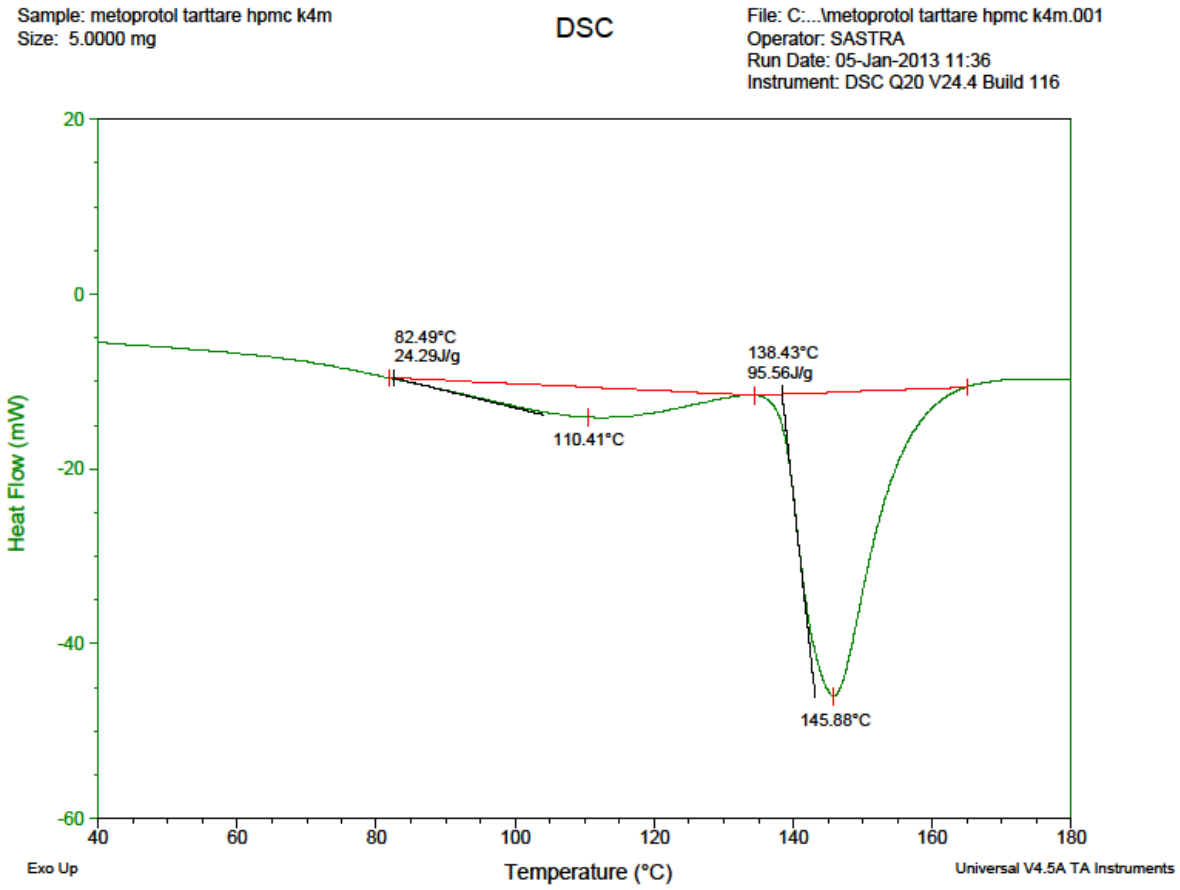


Fig. 9.6: DSC thermo gram of Metoprolol art rate + HPMC K4M

Table 9.6: Data for DSC thermo gram parameters

Sr. No.	DSC thermo gram sample	Onset temperature (°C)	Peak temperature (°C)
1	Metoprolol tartrate	140.94	145.33
2	Metoprolol tartrate+ Carbopol934P	136.58	144.26
3	Metoprolol tartrate+ HPMC K4M	138.43	145.88

DSC thermo gram showed that there was no any major difference in onset temperature and peak temperature, when compared with thermo gram of pure drug. So, it was found that no interaction between drug and polymers.

9.3. PHARMACOTECHNICAL CHARACTERISTICS OF THE FILMS:

9.3.1. Dimension (Thickness and diameter)

Thickness and diameter specifications may be set on an individual product basis. There were no marked variations in the thickness and diameter of films within each formulation indicating uniform behavior of film throughout the sealing process. The size (diameter) and thickness of the films of all formulations were reported in Table 9.7.

9.3.2. Determination of Weight of films

From each batch randomly three films were selected and weighed. The weight variations of films of all formulations were reported in Table 9.7.

9.3.3. Folding endurance:

Use of less amount of plasticizer was observed to cause brittleness in the medicated discs, but use of greater amount of plasticizer (1mL plasticizer per 10 mL) displayed little opaqueness and good folding endurance. The values were reported in the table 9.7

Table 9.7: Pharmacotechnical evaluation of buccoadhesive films

Formulation Code	Dimension		Weight of the film (mg) * ± SD	Folding endurance * ± SD
	Diameter (cm)* ± SD	Thickness (mm)* ± SD		
BF1	1.98 ± 0.08	1.03 ± 0.017	231.3 ± 0.67	310.66 ± 1.15
BF2	2.04 ± 0.048	0.92 ± 0.060	279.2 ± 0.05	315.33± 4.93
BF3	2.04 ± 0.048	1.57 ± 0.479	244.8 ± 0.44	334.33 ± 11.59
BF4	2.06 ± 0.048	1.47 ± 0.110	329.8 ± 0.94	340.00 ± 4.00
BF5	2.02 ± 0.074	1.78 ± 0.064	262.4± 0.01	353.00 ± 07.81
BF6	2.00 ± 0.063	1.84 ± 0.094	304.8 ± 0.05	343.3 ± 13.65
BF7	2.00 ± 0.063	1.06 ± 0.015	358.4 ± 0.95	359.00 ± 13.47
BF8	2.02 ± 0.074	1.14 ± 0.191	272.4 ± 0.77	347.00±7.54
BF9#	2.08±0.054	1.92± 0.052	258.5±0.04	350.6±10.26

All the values were expressed as mean± S.D.,*n=3,#extra design check point formulation

9.3.4 In-Vitro Swelling Study:-

Table 9.8: *In-vitro* swelling study

Sr.No.	Formulation code	Swelling index(%)
1	BF1	29.72±0.660
2	BF2	28.86±0.890
3	BF3	39.06 ±0.690
4	BF4	42.39±0.400
5	BF5	21.41±0.370
6	BF6	32.70±0.670
7	BF7	41.30±0.130
8	BF8	50.13±0.420
9	BF9	45.00±1.040

All the values were expressed as mean± S.D.,*n=3

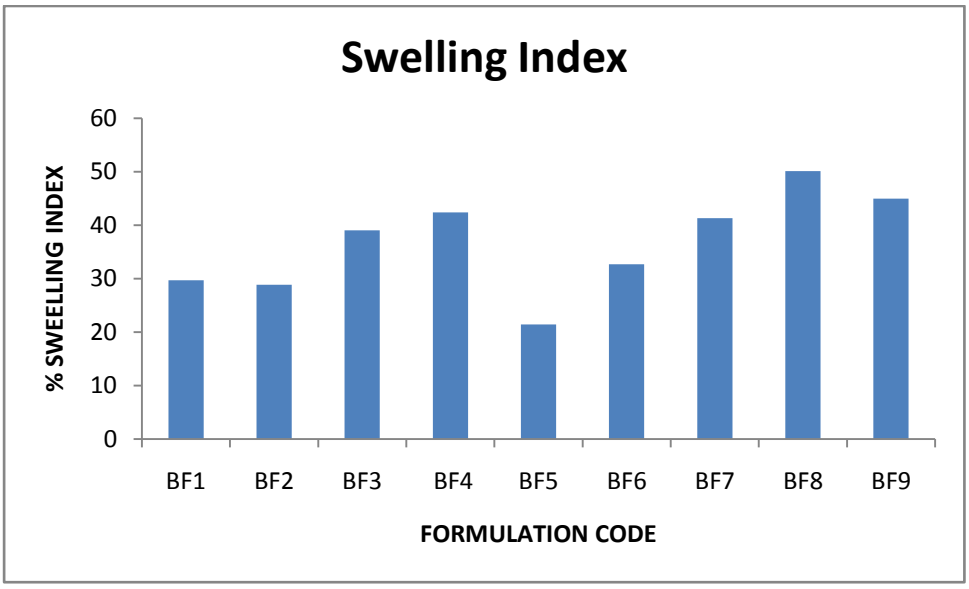


Fig.9.7: *In-vitro* swelling index

The bioadhesion and drug release profile are dependent upon swelling behavior of the Film. Swelling index was calculated with respect to time. Swelling index increased as the weight gain by the Film increased proportionally with the rate of hydration. The films started to swell within 5 min due to presence of swellable HPMC K4M and carbopol 934P, and maximum degree of swelling was observed after 30 min.

The films containing high level of carbopol 934P (BF3, BF4, BF7, BF8) exhibited higher degree of swelling as compared to films containing low level of carbopol 934P (BF1, BF2, BF5, BF6). This is be due to the concentration based swelling behavior of carbopol 934P available for swelling, more will be the swelling index which is beneficial for buccoadhesion. Swelling phenomenon of the polymers makes strong secondary hydrogen bonding with buccal mucosa and thus results in mucoadhesion. Swelling results in the formation of thick swollen mass which provide unidirectional release of drug in sustained manner.

9.3.5. Surface pH Study:-

Table 9.9: Surface pH of Buccoadhesive Film

Sr.No.	Formulation code	Surface pH*
1	BF1	7.04±0.047
2	BF2	6.84± 0.181
3	BF3	6.31 ±0.157
4	BF4	6.23 ±0.080
5	BF5	6.98 ±0.080
6	BF6	6.50 ±0.294
7	BF7	6.02 ±1.000
8	BF8	6.84 ±0.008
9	BF9	6.04 ±0.294

*All the values are expressed as mean± SD, n=3.

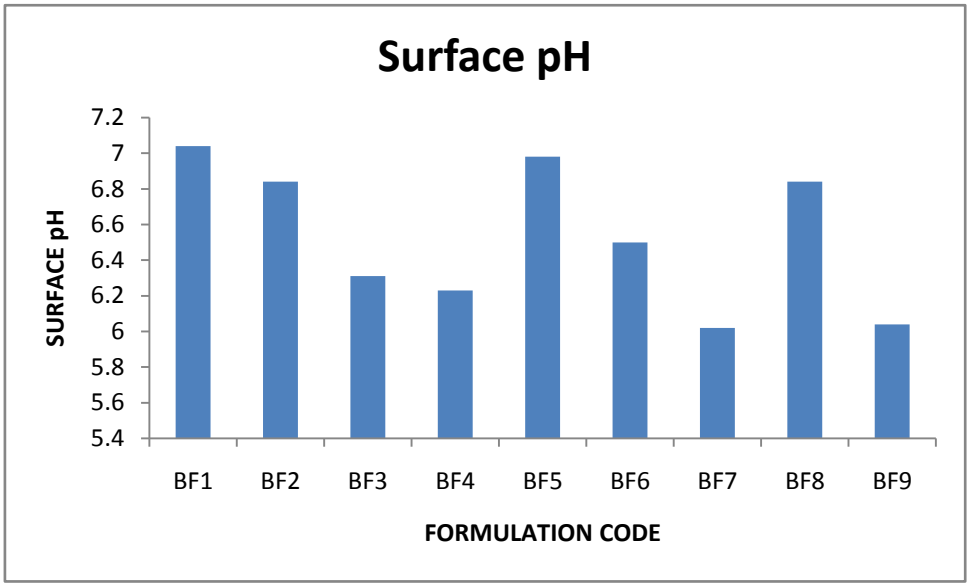


Fig. 9.8: Surface pH of Buccoadhesive Film

The results given in the table and its graphical representation showed that the surface pH of all the Film was within the range of 6.02±0.157 to 7.23±0.080.

These results indicated that there is no risk of mucosal damage or irritation while administering these formulations on buccal mucosal region.

9.3.6.Content uniformity:-

Table 9.10: Content uniformity of buccal film

Sr.No.	Formulation code	Percentage drug content
1	BF1	99.26±1.03
2	BF2	99.92±0.49
3	BF3	98.93±0.85
4	BF4	98.76±0.28
5	BF5	96.13±1.24
6	BF6	96.95±1.78
7	BF7	98.93±0.49
8	BF8	98.36±1.59
9	BF9	97.97±0.49

All the values are expressed as mean± SD, n=3.

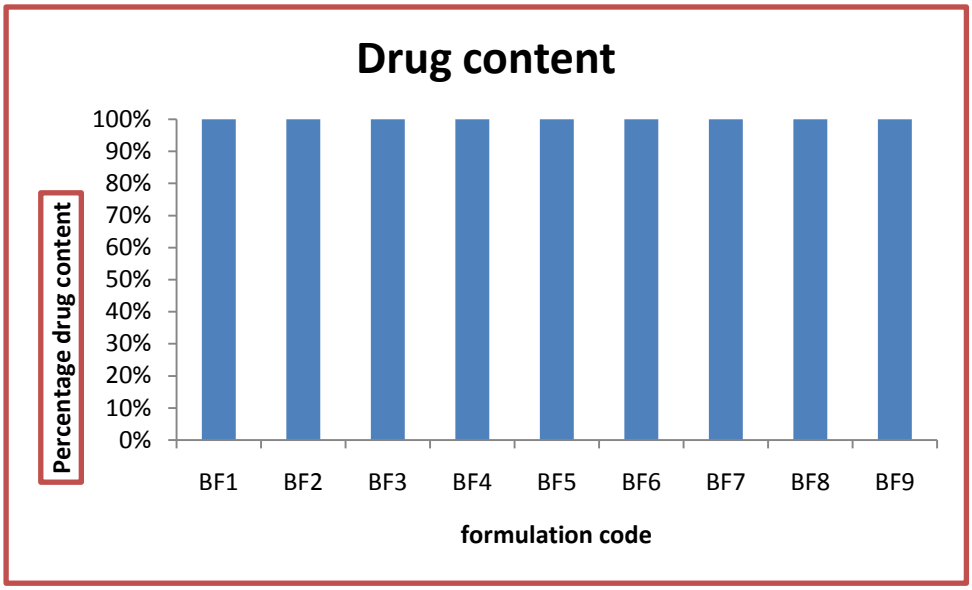


Fig. 9.9: percentage drug content of Buccoadhesive Film

The content uniformity of the prepared buccoadhesive film of the metoprolol tartrate displayed more than 96% drug content. The drug content of prepared buccoadhesive films have within the range of 99.0 to 101.0% as specified in the official monographs.

9.3.7. *Ex-Vivo* Bioadhesion Study:-

Table 9.11: Effect of bioadhesive polymers on bioadhesive strength and force

Sr.No.	Formulation code	Buccoadhesive Strength (gm)	Buccoadhesive Force(N)
1	BF1	32.00±1.000	3.13±0.09
2	BF2	34.00±1.000	3.30±0.01
3	BF3	36.00±1.000	3.52 ±0.09
4	BF4	35.00±1.000	3.30±0.24
5	BF5	32.00±1.520	3.16±0.14
6	BF6	31.00±1.000	3.03±0.19
7	BF7	38.00±2.000	3.72±0.19
8	BF8	39.00±3.200	3.82±0.31
9	BF9	31.00±2.600	3.03±0.25

All the values are expressed as mean± SD, n=3.

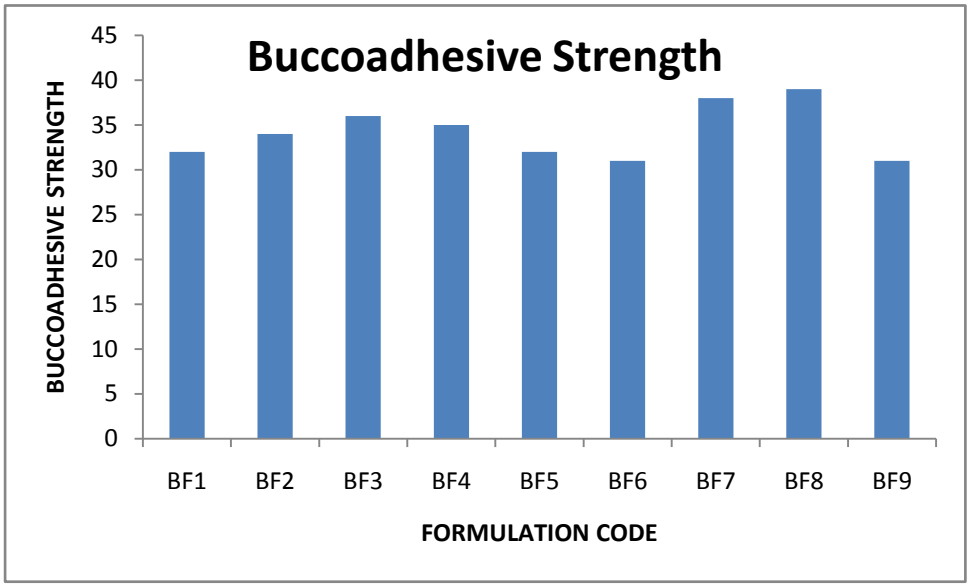


Fig. 9.10: Effect of Bioadhesive polymers on Bioadhesive strength

The results of *ex-vivo* buccoadhesive strength for metoprolol tartrate buccal films are shown in table 9.11. The formulations (BF1 to BF8) exhibited bucco

adhesion strength of 31.00±1.00 to 36.00±1.00 gm and the cut off value for buccoadhesion of a dosage form is 32gm. Thus BF1 was rejected and the rest of the formulations containing high level of carbopol 934 P (BF3,BF4,BF7,BF8) exhibited higher buccoadhesive strength than BF2,BF5,BF6 formulation which may be due to surface adhesion phenomenon as well as due to formation of secondary hydrogen bonds with mucosa as a result of rapidswelling of carbopol 934P.B uccoadhesion is also regulated by the addition of HPMC K4M. It has synergistic effect on buccoadhesiove strength over carbopol 934P, correspondingly BF7,BF8 displayed highest buccoadhesive strength.

9.3.8. *Ex-Vivo* Residence Time:-

Table 9.12: Residence time of Buccoadhesive Film

Sr.No.	Formulation code	Residence time (hours)
1	BF1	07.06±0.66
2	BF2	10.41±0.07
3	BF3	12.34 ±0.11
4	BF4	12.81±0.57
5	BF5	11.96±0.48
6	BF6	11.68±0.54
7	BF7	13.03±0.55
8	BF8	13.05±0.03
9	BF9	10.68±0.51

*All the values are expressed as mean± SD, n=3

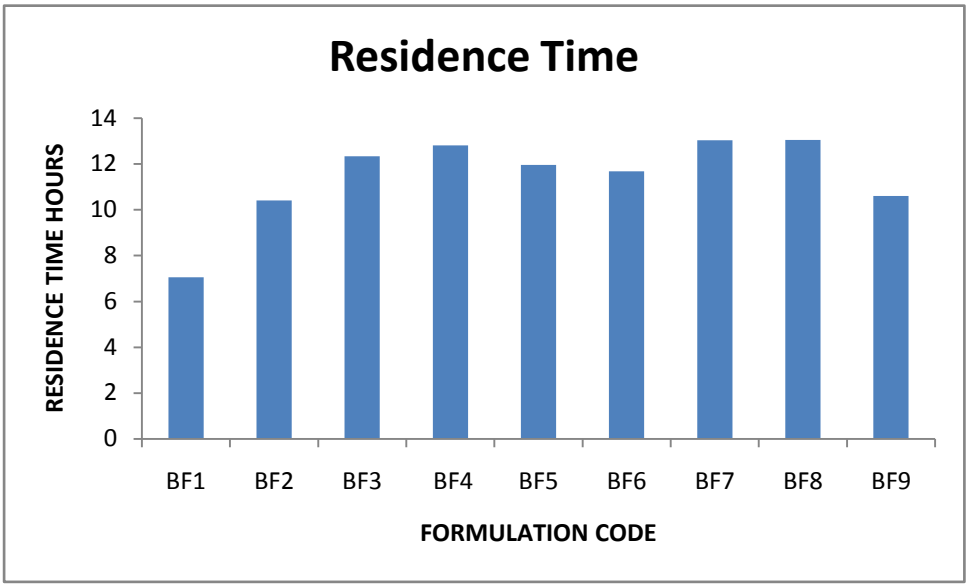


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Fig. 9.11: Data for the *ex-vivo* residence time

The *ex-vivo* Residence time was examined after application of the buccal films on freshly cut goat buccal mucosa. The result showed in Table 9.12, revealed that the mean adhesion time was increased in the formulation batches containing Carbopol 934P: HPMC K4M combination. This may be due to the flexibility of Carbopol 934 P chains, which easily diffuses and interpenetrates into the mucin and get entangled with that of mucin. The mucoadhesive time on goat buccal mucosa ranged from 7.06 to 12.05 hours. The films containing high level of carbopol 934P (BF3,BF4,BF7,BF8) showed higher residence time of 11.34 to 12.05 hr as films containing low level of carbopol 934P (BF1,BF2,BF5,BF6) that show residence time of 7.06 to 10.68hr.This may due to surface adhesion phenomenon as well as due to formation of secondary hydrogen bonds with goat buccal mucosa as a result of rapid swelling of carbopol 934P. BF7 and BF8 show higher residence time than BF3 and BF4 due to presence of HPMC K4M at high level. Hence it can be concluded that ex-vivo residence time increased with increase in the HPMC concentration in the formulation.

9.3.9. *In-Vitro* Drug Release Study:-

Table 9.13: Drug release data of formulation BF1

Sr. No.	Time (hours)	Cumulative % drug release
1	0	0.000±0.00
2	0.5	23.15±1.3058
3	1	26.84±1.3887
4	2	31.156±1.0319
5	3	36.793±1.3766
6	4	43.716±1.1168
7	5	53.82±0.9690
8	6	64.66±0.3893
9	7	77.68±1.5326
10	8	92.19±0.6022

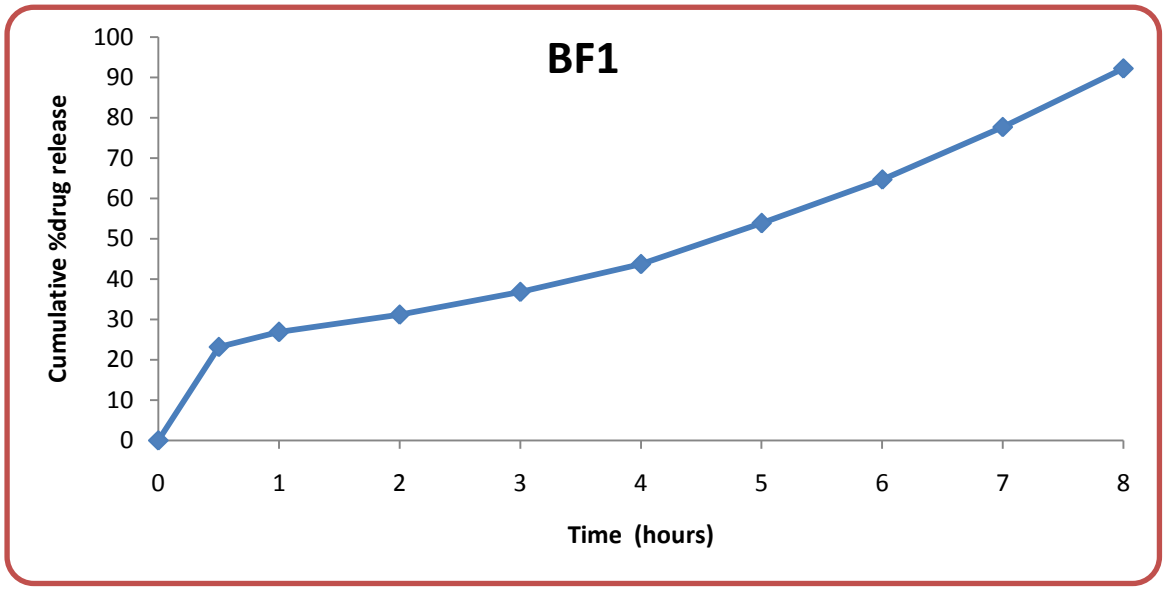


Fig. 9.12: Drug release profile of formulation BF1

Table 9.14: Drug release data of formulation BF2

Sr. No.	Time (hours)	Cumulative % drug release
1	0	0.000±0.00
2	0.5	20.69±0.62
3	1	24.57±0.37
4	2	31.03±0.90
5	3	34.43±0.76
6	4	42.96±0.29
7	5	53.34±0.32
8	6	63.00±0.24
9	7	75.55±0.81
10	8	89.37±0.41

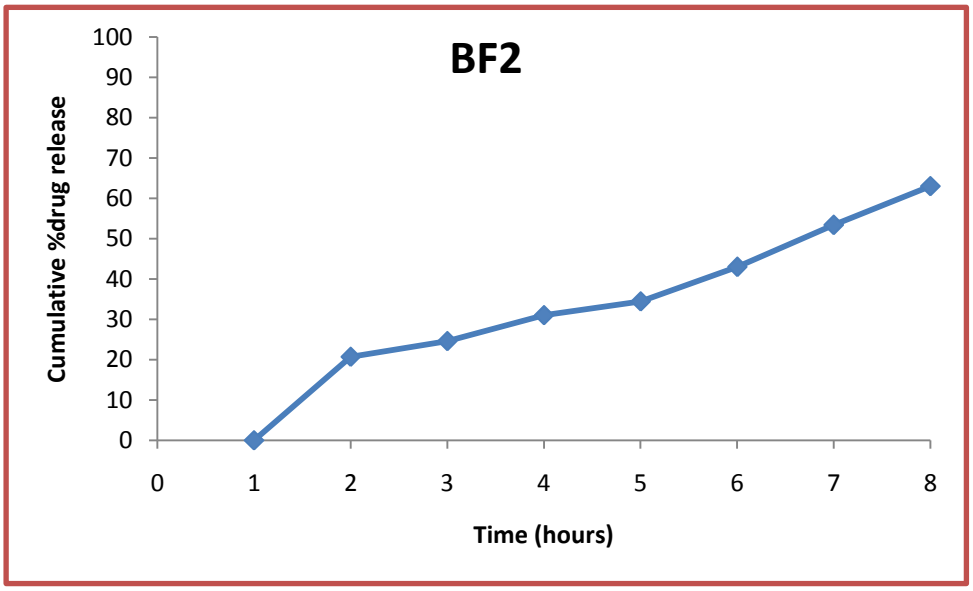


Fig. 9.13: Drug release profile of formulation BF2

Table 9.15: Drug release data of formulation BF3

Sr. No.	Time (hours)	Cumulative % drug release
1	0	0.000±0.00
2	0.5	20.11±0.70
3	1	24.64±0.42
4	2	36.18±0.19
5	3	41.40±1.07
6	4	51.56±0.30
7	5	56.41±0.35
8	6	63.49±0.45
9	7	71.88±0.47
10	8	80.26±0.67

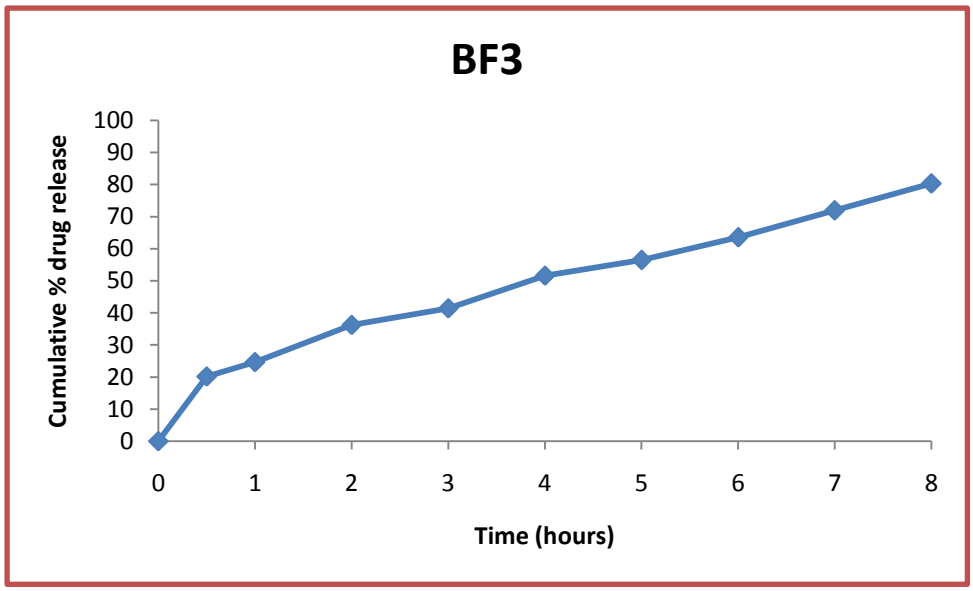


Fig. 9.14: Drug release profile of formulation BF3

Table 9.16 Drug release data of formulation BF4

Sr. No.	Time (hours)	Cumulative % drug release
1	0	0.000±0.00
2	0.5	21.6±0.37
3	1	25.74±0.49
4	2	37.49±0.17
5	3	41.85±0.44
6	4	52.22±0.20
7	5	57.41±0.58
8	6	65.21±0.37
9	7	72.81±0.14
10	8	84.29±0.46

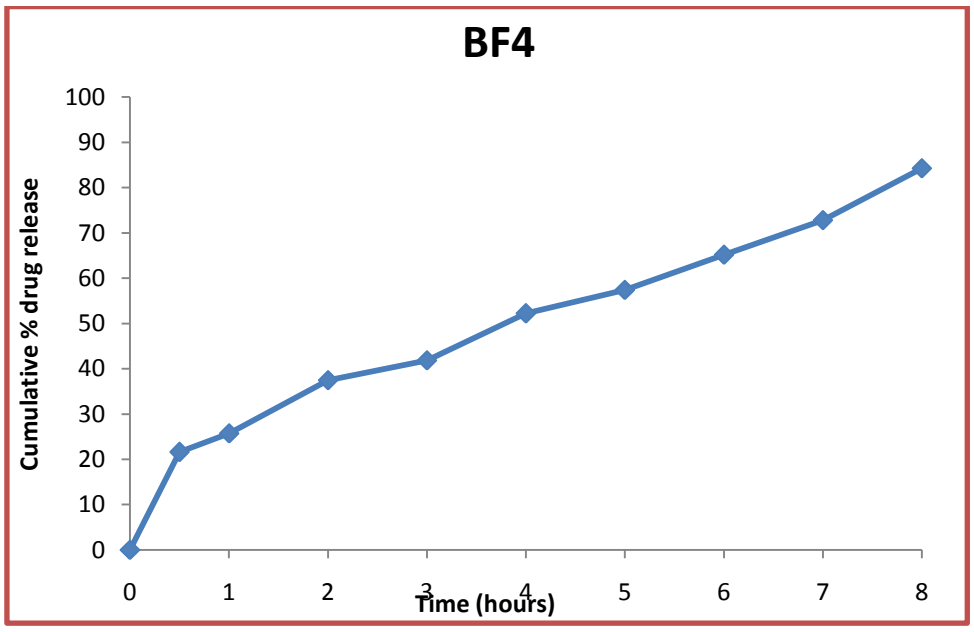


Fig. 9.15: Drug release profile of formulation BF4

Table 9.17: Drug release data of formulation BF5

Sr. No.	Time (hours)	Cumulative % drug release
1	0	0.000±0.00
2	0.5	17.19±0.32
3	1	22.97±0.50
4	2	33.60±0.48
5	3	39.70±0.09
6	4	48.62±0.42
7	5	54.41±0.58
8	6	61.54±0.57
9	7	70.35±0.48
10	8	77.54±0.51

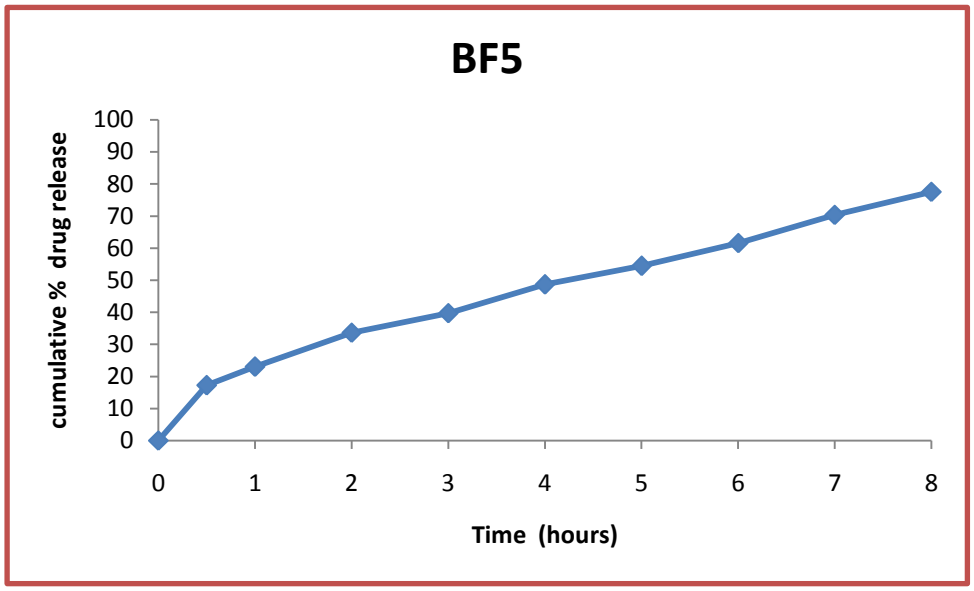


Fig. 9.16: Drug release profile of formulation BF5

Table 9.18: Drug release data of formulation BF6

Sr. No.	Time (hours)	Cumulative % drug release
1	0	0.000±0.00
2	0.5	17.52±0.13
3	1	22.36±0.32
4	2	32.94±0.32
5	3	38.39±0.38
6	4	47.42±0.26
7	5	52.2±0.20
8	6	61.13±0.13
9	7	68.95±0.42
10	8	74.80±0.54

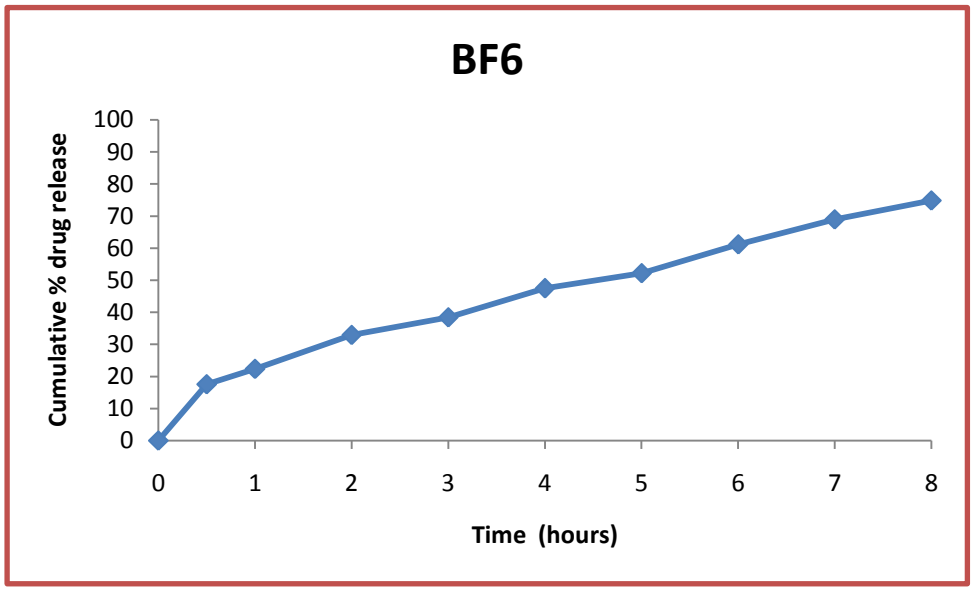


Fig. 9.17: Drug release profile of formulation BF6

Table 9.19: Drug release data of formulation BF7

Sr. No.	Time (hours)	Cumulative % drug release
1	0	0.000±0.00
2	0.5	17.52±0.05
3	1	22.03±0.43
4	2	33.01±0.31
5	3	37.82±0.32
6	4	45.76±0.27
7	5	52.26±0.24
8	6	59.87±0.25
9	7	68.93±0.35
10	8	73.56±0.42

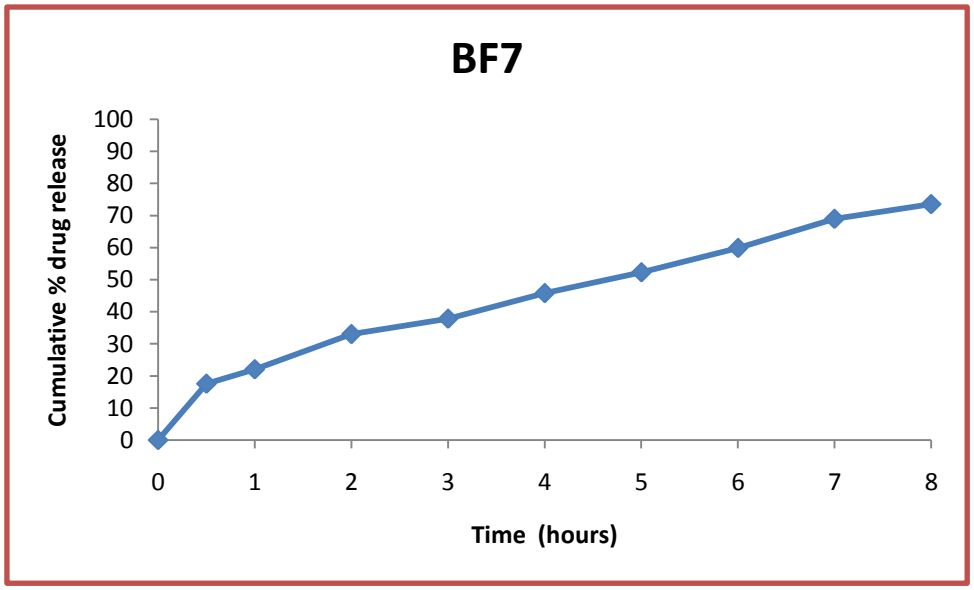


Fig. 9.18: Drug release profile of formulation BF7

Table 9.20: Drug release data of formulation BF8

Sr. No.	Time (hours)	Cumulative % drug release
1	0	0.000±0.00
2	0.5	14.92±0.82
3	1	19.91±0.57
4	2	31.69±0.21
5	3	36.42±0.40
6	4	44.38±0.62
7	5	48.54±0.39
8	6	57.88±0.66
9	7	66.71±0.88
10	8	71.29±0.62

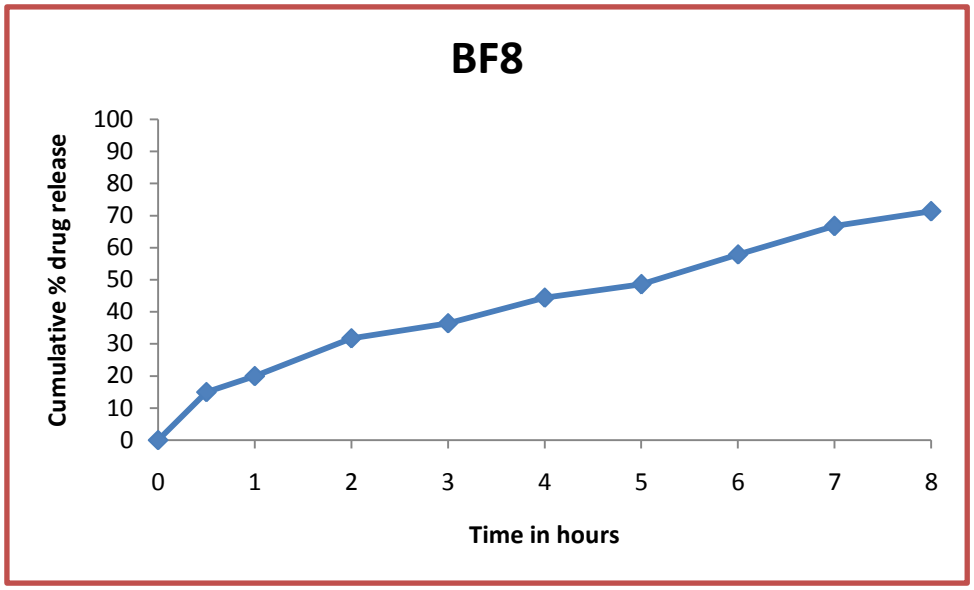


Fig. 9.19: Drug release profile of formulation BF8

Table 9.21: Drug release data of formulation BF9

Sr. No.	Time (hours)	Cumulative % drug release
1	0	0.000±0.00
2	0.5	20.07±0.07
3	1	23.93±0.18
4	2	34.54±0.19
5	3	40.43±0.40
6	4	47.28±0.36
7	5	51.93±0.25
8	6	61.48±0.51
9	7	69.80±0.52
10	8	76.31±0.51

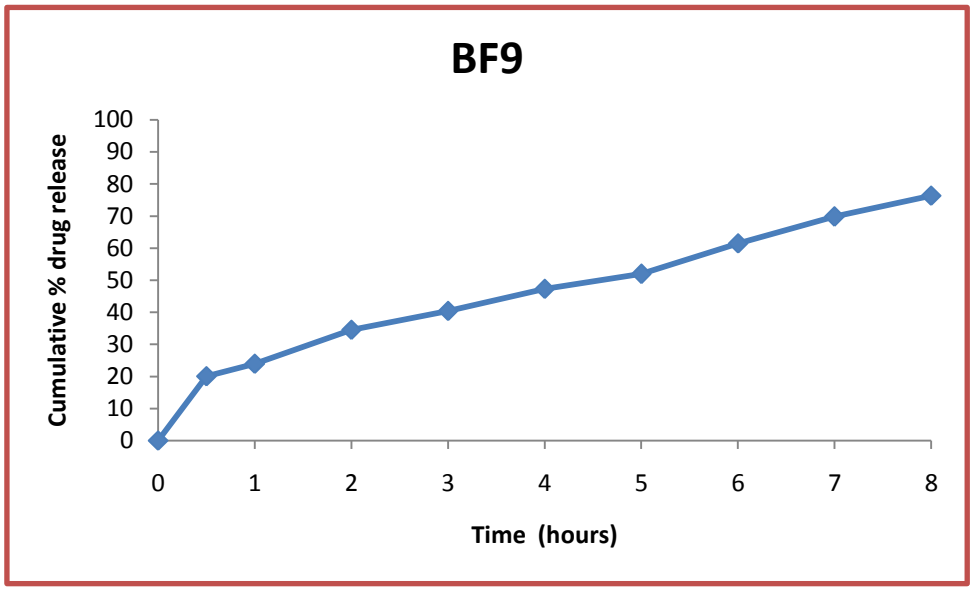


Fig. 9.20: Drug release profile of formulation BF9

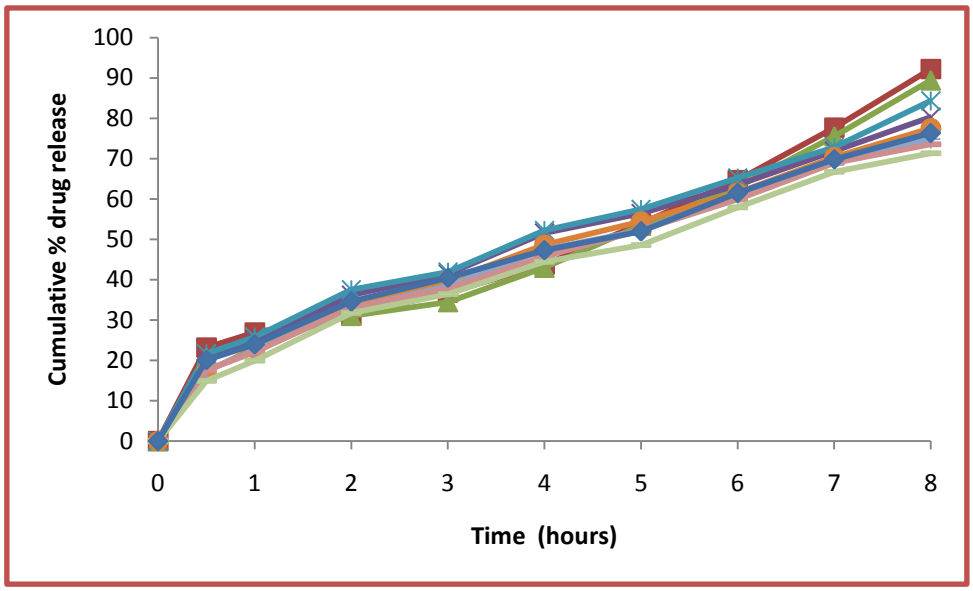


Fig. 9.21: Comparative Drug release profile of all Buccoadhesive Film.

In-vitro drug release studies revealed that the release of Metoprolol tartrate from different formulations varies with the characteristics and composition of matrix forming polymers as shown in figures 9.12 to 9.20. Films containing low level of HPMC K4M (BF1,BF2,BF3,BF4)displayed higher in-vitro drug release (92.19± 0.60 to 80.26±0.67)than formulations containing higher level of HPMC K4M (BF5,BF6,BF7,BF8)that displayed only (71.29±0.62 to 77.54±0.51) drug release after 8hr which may due to increase viscosity offered by the gelling of the hydrophilic HPMC K4M polymer. The increased viscosity of formulation resulted in a corresponding decrease viscosity of by the gelling of the hydrophilic HPMC K4M polymer. The increased viscosity of formulation resulted in a corresponding decrease in the release. A similar observation has been obtained by other reference drug. Where as decrease in metoprolol tartrate release was obtained on increasing the concentration of HPMC and carbopol 934P. Though highest %CDR of 92.19 ±0.60% at 8^{th} was recorded for BF1, the formulation was rejected based on poor ex-

vivo residence time, thus BF4 was considered as second best formulation in terms of %CDR (84.29%)and least by BF8 (71.29%) which is showing an inverse relation between concentration of HPMC K4M and in-vitro drug release. In formulations BF1, BF2, BF3, BF4 drug release with increasing the concentration of carbopol 934 P. Since carbopol 934P is insoluble in stimulated saliva and swelling behavior of carbopol 934P is attributed to unchanged COOH group that get hydrated by forming hydrogen bonds on imbibing with water and therefore extending polymer chain. It was observed that films containing combination of high levels of both carbopol934P and HPMCK4M exhibited delayed drug release indicating better matrix characteristics. Strong matrix integrity inhibits the entry of dissolution media and delays the dissolution of drug.

9.3.10. In-Vitro buccal permeation:-

Table 9.22: Drug permeation data of formulation BF1

Sr. No.	Time (hours)	Cumulative % drug permeation
1	0	0.000±0.00
2	1	4.13±0.37
3	2	13.85±0.87
4	3	16.03±2.62
5	4	21.88±0.30
6	5	23.65±0.21
7	6	29.64±0.190
8	7	35.93±1.20
9	8	39.60±1.50

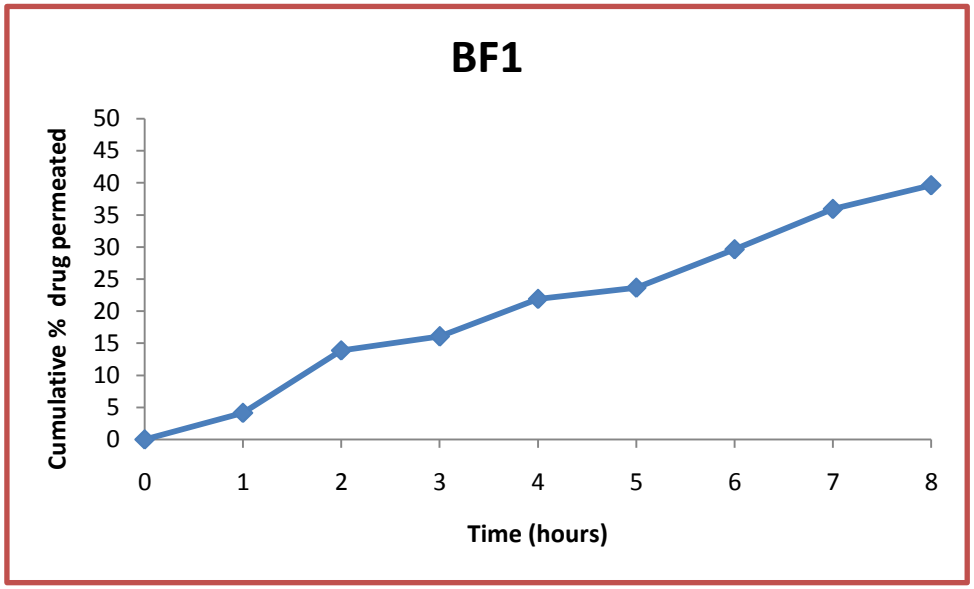


Fig. 9.22: Drug permeation profile of formulation BF1

Table 9.23: Drug permeation data of formulation BF2

Sr. No.	Time (hours)	Cumulative % drug permeation
1	0	0.000±0.00
2	1	5.59±0.15
3	2	11.18±0.09
4	3	16.92±0.10
5	4	22.57±0.06
6	5	27.59±0.46
7	6	32.96±1.10
8	7	36.41±0.11
9	8	38.62±0.18

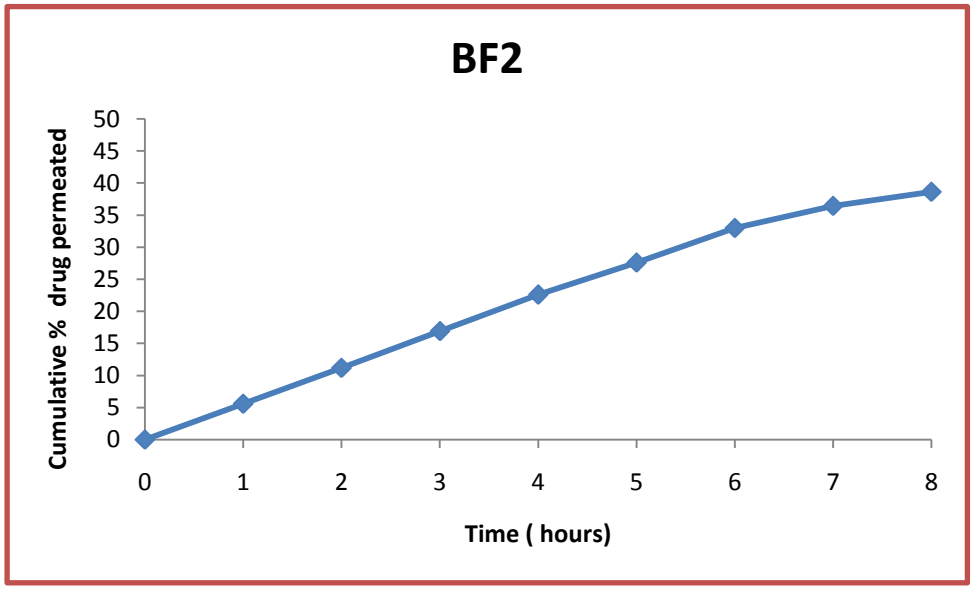


Fig. 9.23: Drug permeation profile of formulation BF2

Table 9.24: Drug permeation data of formulation BF3

Sr. No.	Time (hours)	Cumulative % drug permeation
1	0	0.000±0.00
2	1	6.32±0.37
3	2	11.36±0.27
4	3	17.65±0.20
5	4	22.81±0.24
6	5	30.98±2.20
7	6	35.31±1.164
8	7	38.10±0.94
9	8	40.07±0.06

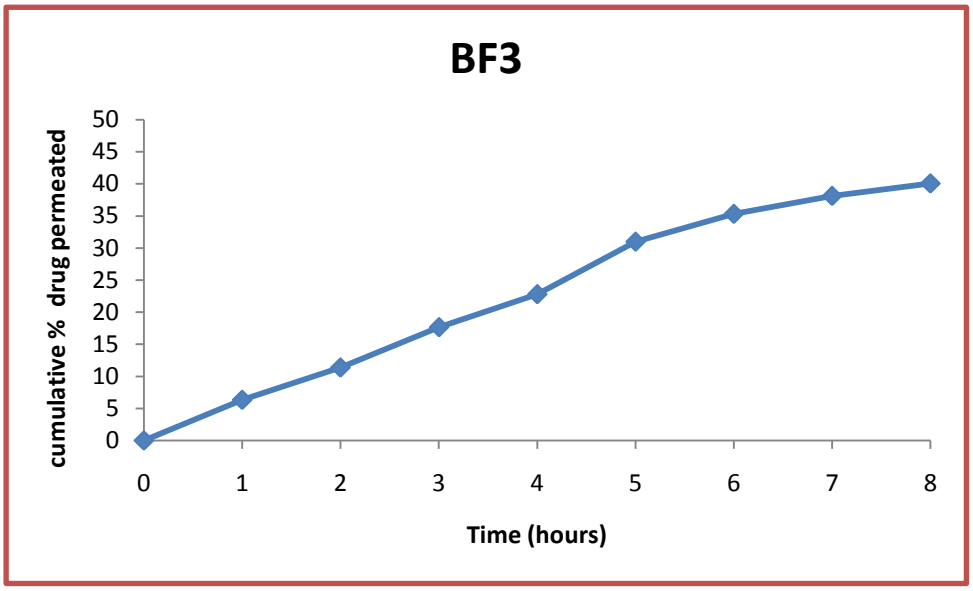


Fig. 9.24: Drug permeation profile of formulation BF3

Table 9.25:Drug permeation data of formulation BF4

Sr. No.	Time (hours)	Cumulative % drug permeation
1	0	0.000±0.00
2	1	07.08±0.05
3	2	13.25±1.16
4	3	17.00±0.14
5	4	22.91±1.09
6	5	27.72±1.38
7	6	32.59±0.37
8	7	37.51±0.90
9	8	42.68±1.35

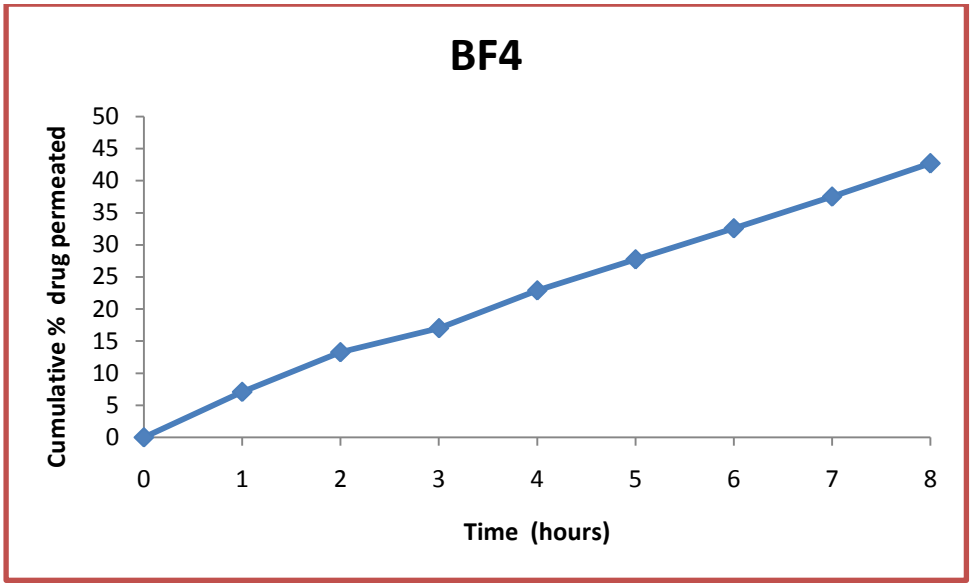


Fig. 9.25: Drug permeation profile of formulation BF4

Table 9.26: Drug permeation data of formulation BF5

Sr. No.	Time (hours)	Cumulative % drug permeation
1	0	0.000±0.00
2	1	11.97±0.84
3	2	15.04±0.71
4	3	19.09±0.12
5	4	22.17±1.8
6	5	24.63±0.32
7	6	26.62±1.38
8	7	34.61±0.75
9	8	36.64±0.18

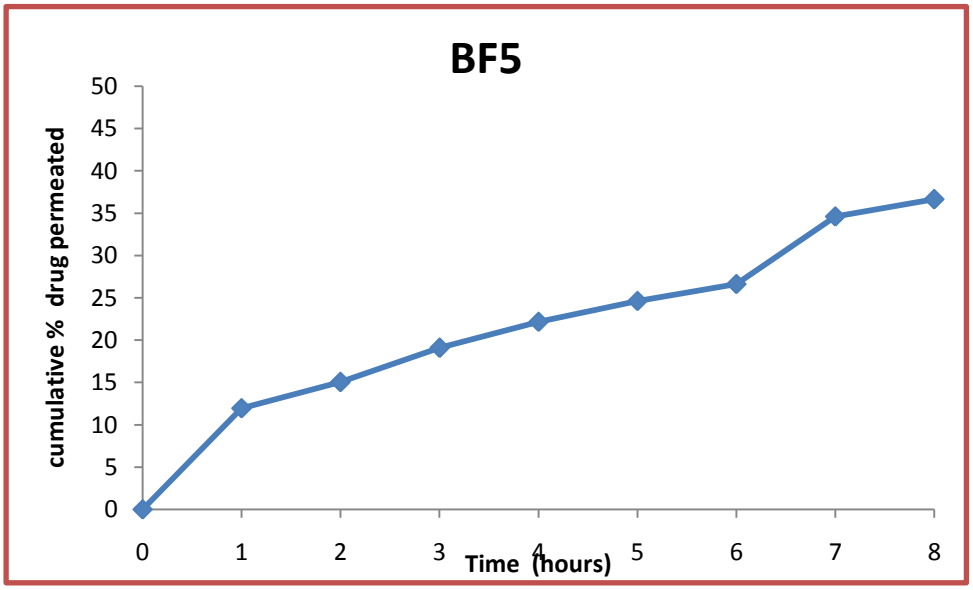


Fig. 9.26: Drug permeation profile of formulation BF5

Table 9.27: Drug permeation data of formulation BF6

Sr. No.	Time (hours)	Cumulative % drug permeation
1	0	0.000±0.00
2	1	12.32±0.23
3	2	14.86±0.55
4	3	19.63±0.05
5	4	21.84±0.71
6	5	24.36±0.43
7	6	32.44±3.31
8	7	34.79±1.62
9	8	38.40±0.70

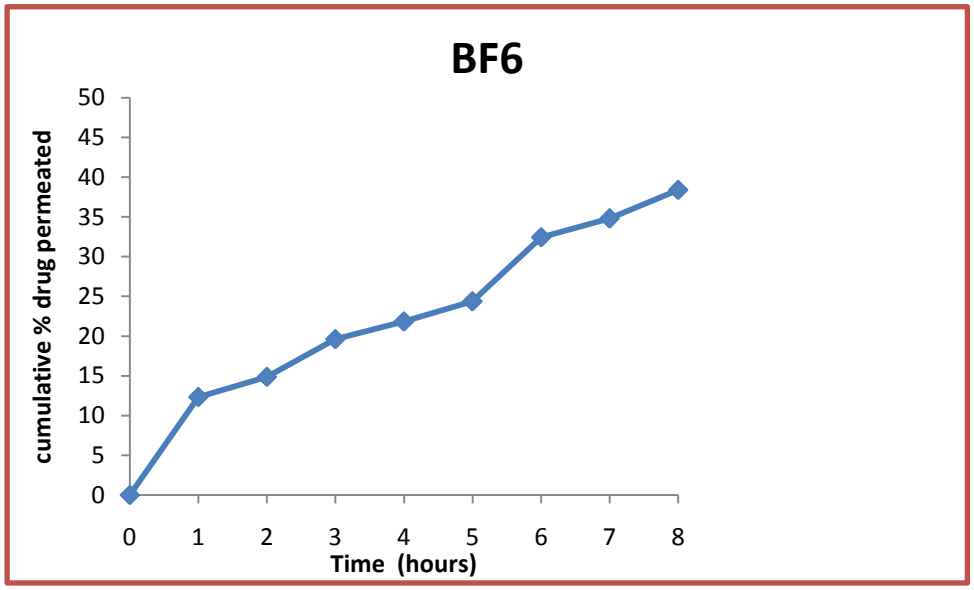


Fig. 9.27: Drug permeation profile of formulation BF6

Table 9.28: Drug permeation data of formulation BF7

Sr. No.	Time (hours)	Cumulative % drug permeation
1	0	0.000±0.00
2	1	09.96±0.91
3	2	16.01±0.23
4	3	20.02±0.23
5	4	22.17±0.22
6	5	30.77±0.23
7	6	35.85±0.43
8	7	37.04±0.27
9	8	41.21±0.12

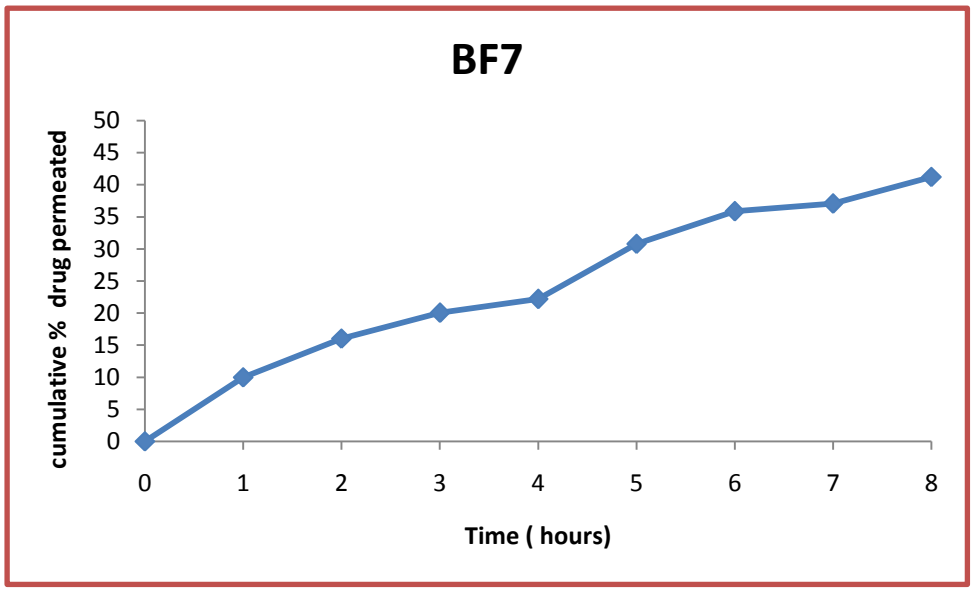


Fig. 9.28: Drug permeation profile of formulation BF7

Table 9.29: Drug permeation of formulation BF8

Sr. No.	Time (hours)	Cumulative % drug permeation
1	0	0.000±0.00
2	1	05.20±0.05
3	2	11.52±1.14
4	3	12.86±0.76
5	4	27.92±0.55
6	5	32.26±0.62
7	6	35.68±1.28
8	7	38.92±0.92
9	8	41.84±0.54

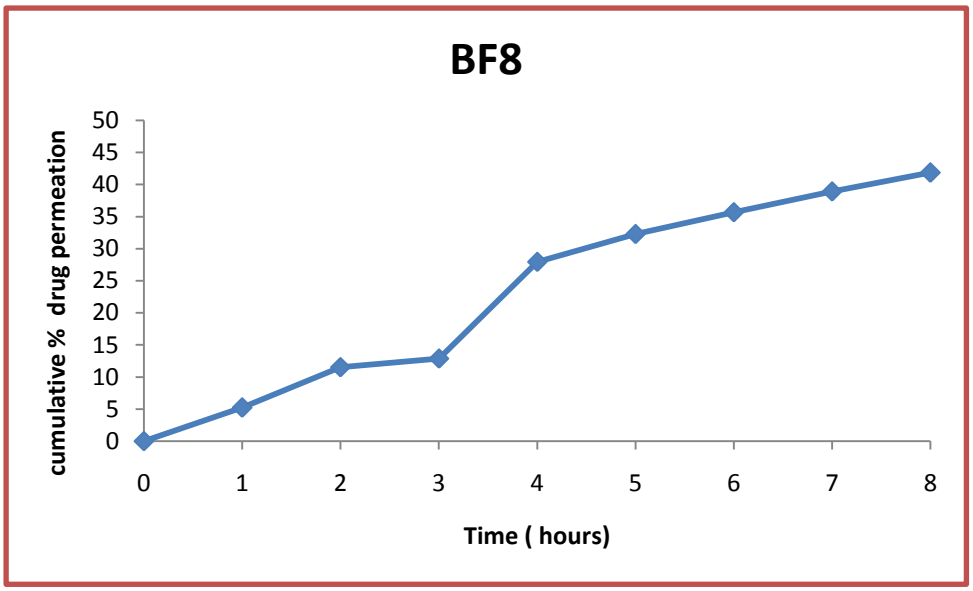


Fig. 9.29: Drug permeation profile of formulation BF8

Table 9.30: Drug permeation data of formulation BF9

Sr. No.	Time (hours)	Cumulative % drug permeation
1	0	0.000±0.00
2	1	05.20±0.05
3	2	08.84±1.57
4	3	11.99±0.10
5	4	27.45±0.37
6	5	31.52±1.00
7	6	33.54±0.00
8	7	33.18±2.30
9	8	37.08±0.69

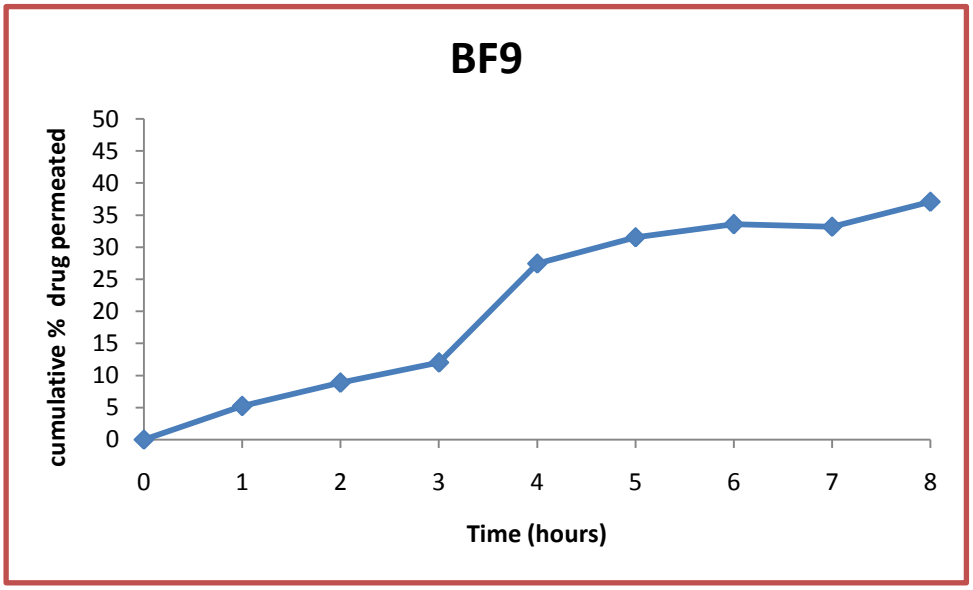


Fig. 9.30: Drug permeation profile of formulation F9

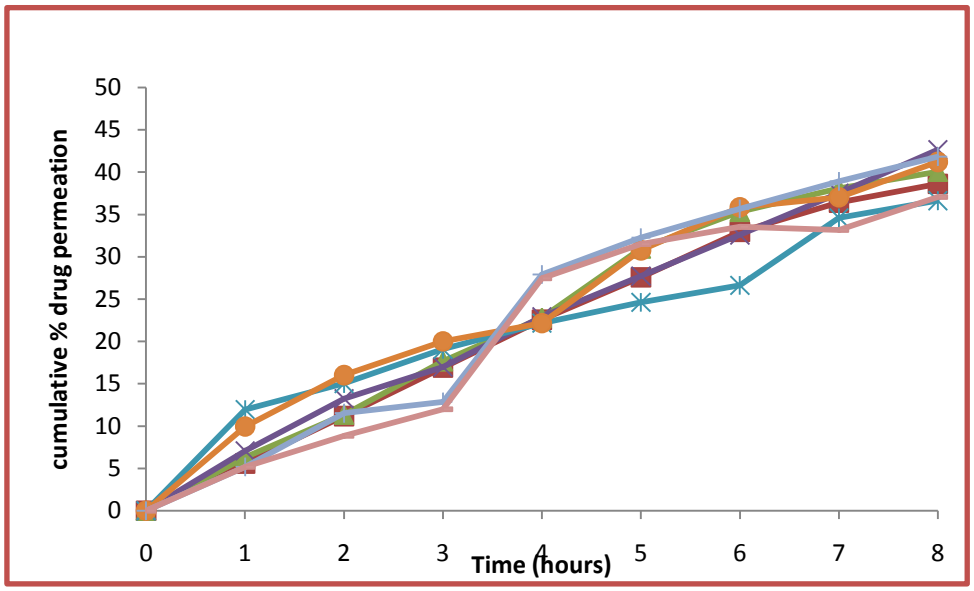


Fig. 9.31: Comparative Drug Permeation profile of all Buccoadhesive Film.

In-vitro drug permeation studies revealed that the release of Metoprolol tartrate from different formulations varies with the characteristics and composition of matrix forming polymers as shown in figures 9.22 to 9.30.

Metoprolol tartrate being hydrophilic with log P value of 1.9 exhibits high permeability buccal mucosa and there is a need to enhance its buccal mucosa and there is a need to enhance its buccal permeation with help of permeation enhancer that causes perturbation and dissolution of paracellular fluid, enhancing its paracellular of transport. Based on this fact, different concentrations of DMSO were tried to improve the permeation of metoprolol tartrate through buccal mucosa. The results suggested that on increasing the concentration of DMSO up to 6%, permeability of drug increased.

In the experimental design, formulation BF2,BF4,BF6 and BF8 containing high level of DMSO showed higher permeation of metoprolol tartrate than formulations BF1,BF3,BF5 and BF7 which is Highlighting the significance of level of

DMSO. Amongst all the films containing high levels of DMSO, the descending order for permeability coefficient was BF8>BF4>BF6>BF2 and it can be concluded that proper formulation optimization is essential.

9.3.11. Histopathological studies:

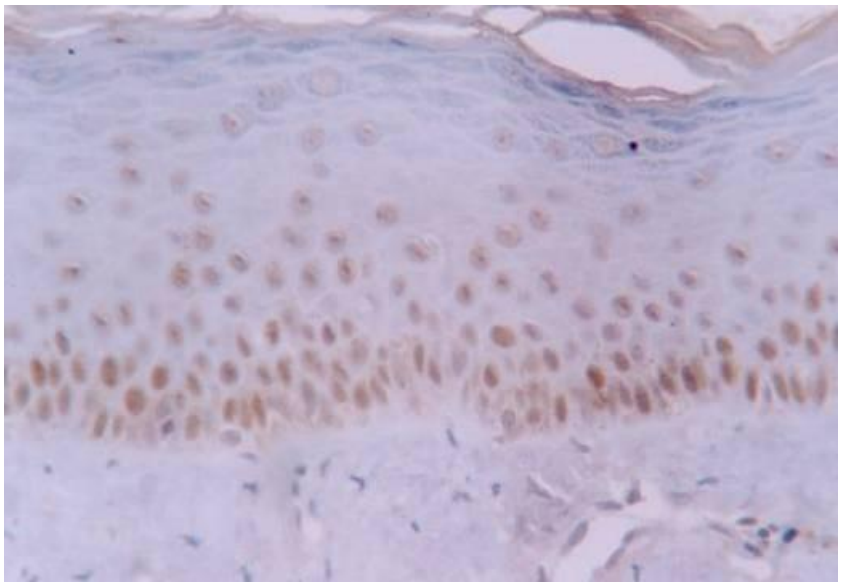


Fig 9.32 Histopathological evaluation of transverse section of goat buccal mucosa treated with optimized formulation BF4

The goat buccal mucosa specimen at the end of permeation study of optimized formulation BF4 was subjected to histopathological evaluation. The microscopic observation of the transverse section showed no damage to the buccal mucosa at cellular level. All the layers mucus,stratum distendum,strtum basale,basal lamina and submucosa were found to be intact establishing the non-toxicity of the optimized film.

9.3.12.Kinetics for Drug Release:

Table 9.31: Drug release kinetic studies of Buccoadhesive Film

Code	Zero order		First order		Higuchi		Korsemeyer-Peppas		Best fit model
	R^2	K_0 (mg/h^{-1})	R^2	K_1 (h^{-1})	R^2	K ($mg\ h^{-1/2}$)	R^2	N	
BF1	0.9561	11.2770	0.9031	0.2191	0.9556	26.67	0.9416	0.0512	Zero order
BF2	0.9597	11.0392	0.9164	0.2065	0.9562	26.09	0.9500	0.0541	Zero order
BF3	0.9240	6.9175	0.9814	0.1847	0.9934	26.19	0.9924	0.1276	Higuchi
BF4	0.9235	11.0773	0.9723	0.1964	0.9904	27.03	0.9877	0.1116	Higuchi
BF5	0.9363	10.7455	0.9867	0.1753	0.9928	25.54	0.9941	0.1262	Peppas
BF6	0.9376	9.3129	0.9887	0.1660	0.9924	24.8564	0.9936	0.1247	Peppas
BF7	0.9366	10.0865	0.9868	0.1623	0.9912	24.47	0.9926	0.1232	Peppas
BF8	0.9463	9.6531	0.9882	0.1546	0.9893	23.72	0.9938	0.1286	Peppas
BF9	0.9204	10.5770	0.9826	0.1724	0.9916	25.40	0.9869	0.1198	Higuchi

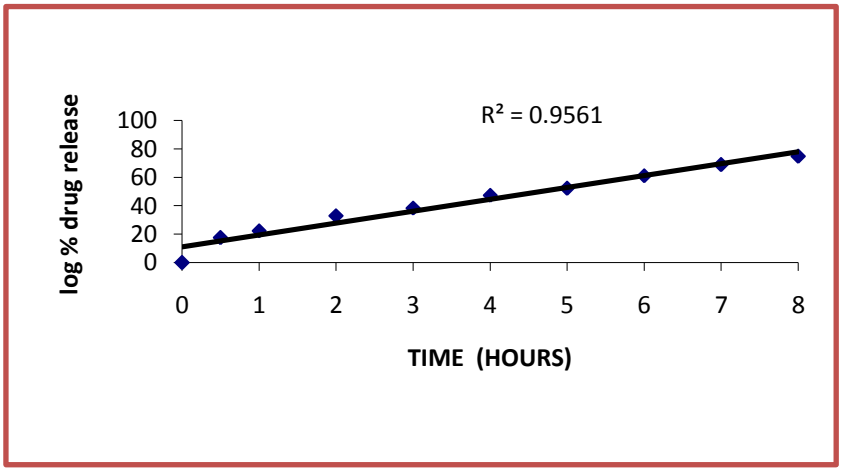


Fig. 9.33: Zero order curve of formulation BF1

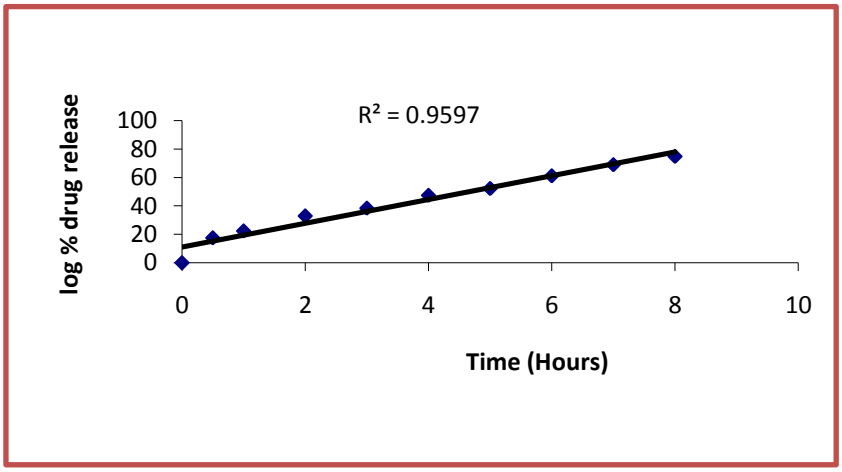


Fig. 9.34: Zero order curve of formulation BF2

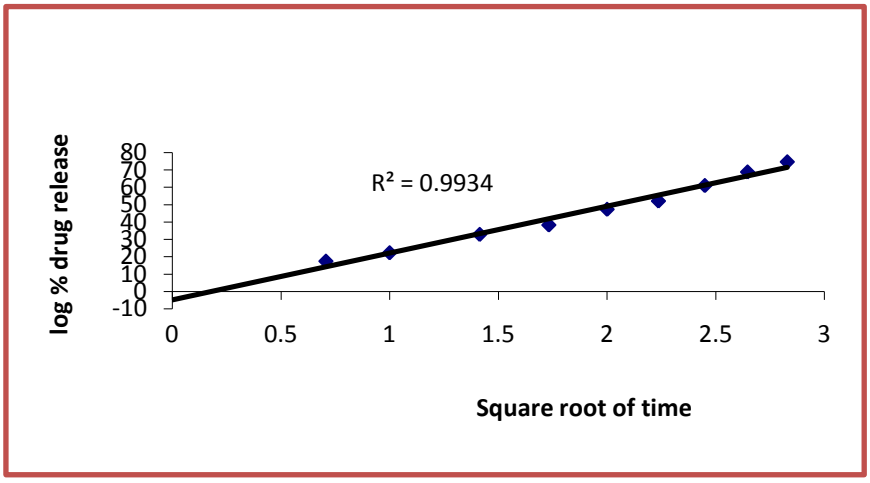


Fig. 9.35: Higuchi plot curve of formulation BF3

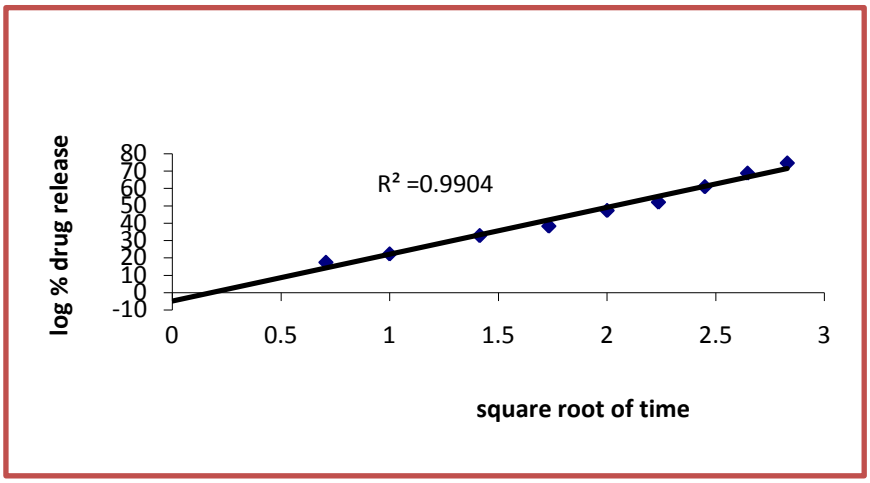


Fig. 9.36: Higuchi plot curve of formulation BF4

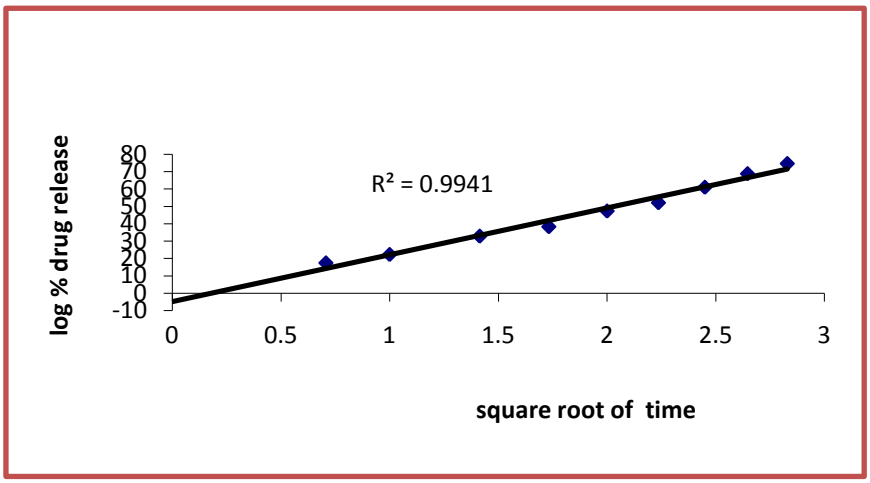


Fig. 9.37: Peppas curve of formulation BF5

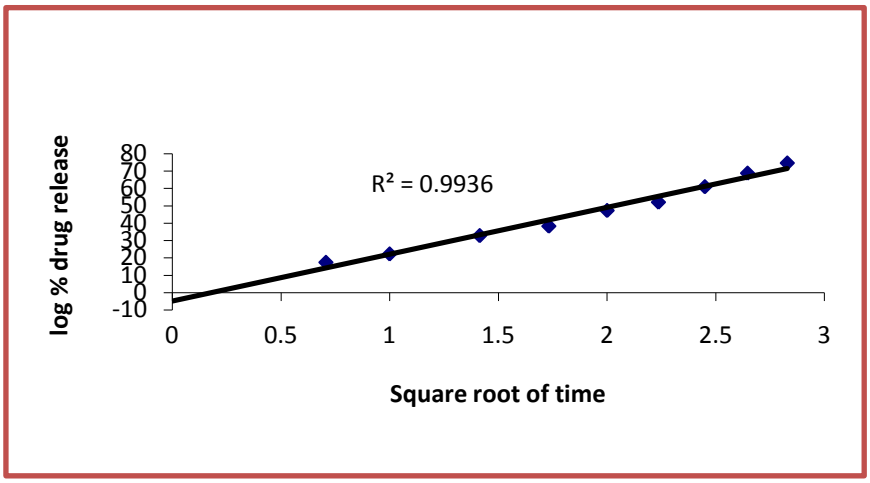


Fig. 9.38: Peppas curve of formulation BF6

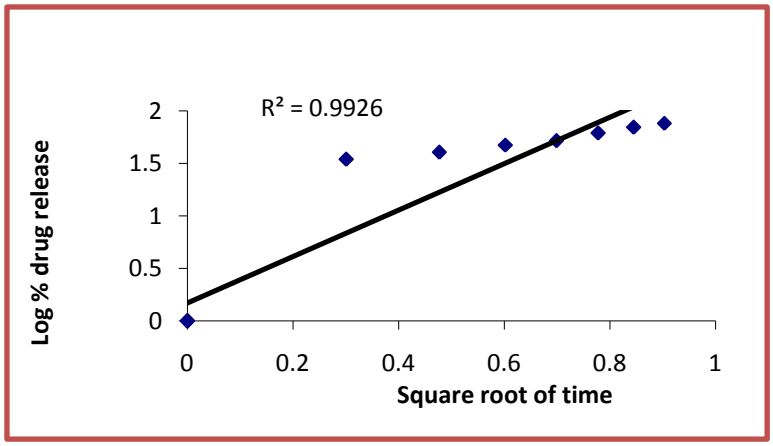


Fig. 9.39: Peppas curve of formulation BF7

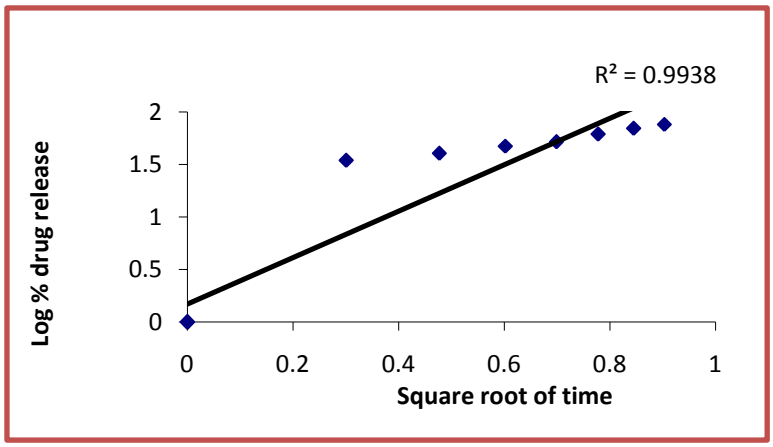


Fig. 9.40: Koresmeyer peppa's curve of formulation BF8

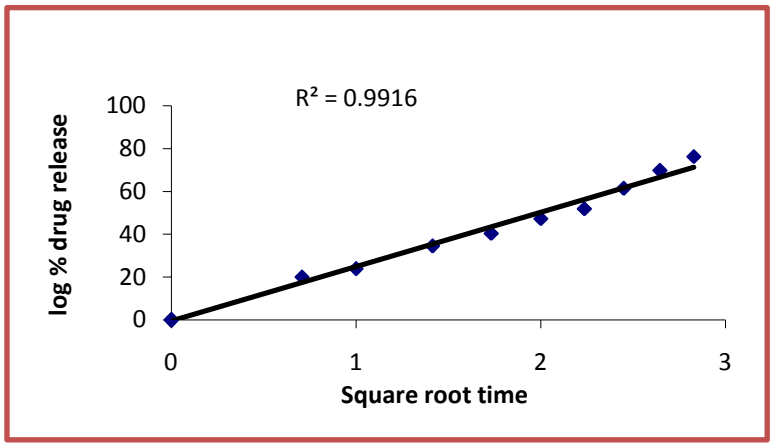


Fig. 9.41: Higuchi plot curve of formulation BF9

Further to characterize the release mechanism of Metoprolol tartrate from buccoadhesive Film, the dissolution data was subjected to the different model such as zero- order, first order, Korsmeyer- peppas and matrix- Higuchi diffusion models.

9.3.13. Statistical analysis of response by design expert software

Based on the results obtained for ex-vivo residence time, %CDR at 8th hr and cumulative %drug permeation at 8th hr, the response polynomial coefficients were determined in order to evaluate each response. Each response coefficient was studied for its statistical significance by Pareto charts as shown in figure. Pareto charts establish 't'value of effect that is studied by two limit lines namely Bonferroni limit line (t value of effect =3.752)and t limit line (t value effect=2.345) coefficients with t value of effect between Bonferroni line are designated as certainly significant coefficients with t value of effect between Bonferroni line and t limit linear termed as coefficients likely to be significant, while t value of effect below the t limit line is statistically insignificant and should be removed from the analysis. The non-significant response coefficients were deleted and the following significant

polynomial response equation(s) for ex-vivo residence time, %CDR at 8^{th} hr and cumulative %drug permeation at 8^{th} hr were generated.

Ex-vivo residence time = $11.17+0.86X_1+(0.524\times[X_2])+[0.495\times(X_3)]+[0.187\times(X_1X_2X_3)]$…….eq 3

%CDR at 8^{th} hr = $80.41–6.85X_1+[–0.964\times(X_2)]+[–0.959\times(X_3)]+[–0.362\times(X_1X_2X_3)]$……..eq 4

Cumulative %drug permeated at 8^{th} hr = $38.63+258X1+[0.663\times(X2)]+[0.634\times(x3)]+[0.240\times(X_1X_2\ X_3)]$……eq 5

Validation of experimental design:

These equations were utilized for validation of the equation of the experimental design. An extra design checkpoint formulation (BF9) was prepared and the predicted values for ex-vivo residence time, %CDR at 8^{th} hr and cumulative %permeation at 8^{th} hr were generated. Experimental values were determined by formulating and evaluating BF9, and close resemblance between predicted and experimental values indicated validity of generated model.

Table 9.32: Evaluation of Extra design check point Formulation BF9 and Optimized formulation BF4

Response parameter	Formulation code	Predicted value	Experimental value	%RSD
%CDR at 8^{th} hr	BF9	76.39	76.31± 0.51	0.08
	BF4	80.41	84.29 ±0.49	3.88
Cumulative %permeation at 8^{th} hr	BF9	36.55	37.05 ±0.69	0.5
	BF4	38.63	42.68 ±1.35	4.05
Ex-vivo residence time (hr)	BF9	10.52	10.68 ±0.51	0.15
	BF4	11.17	11.81 ±0.57	0.74

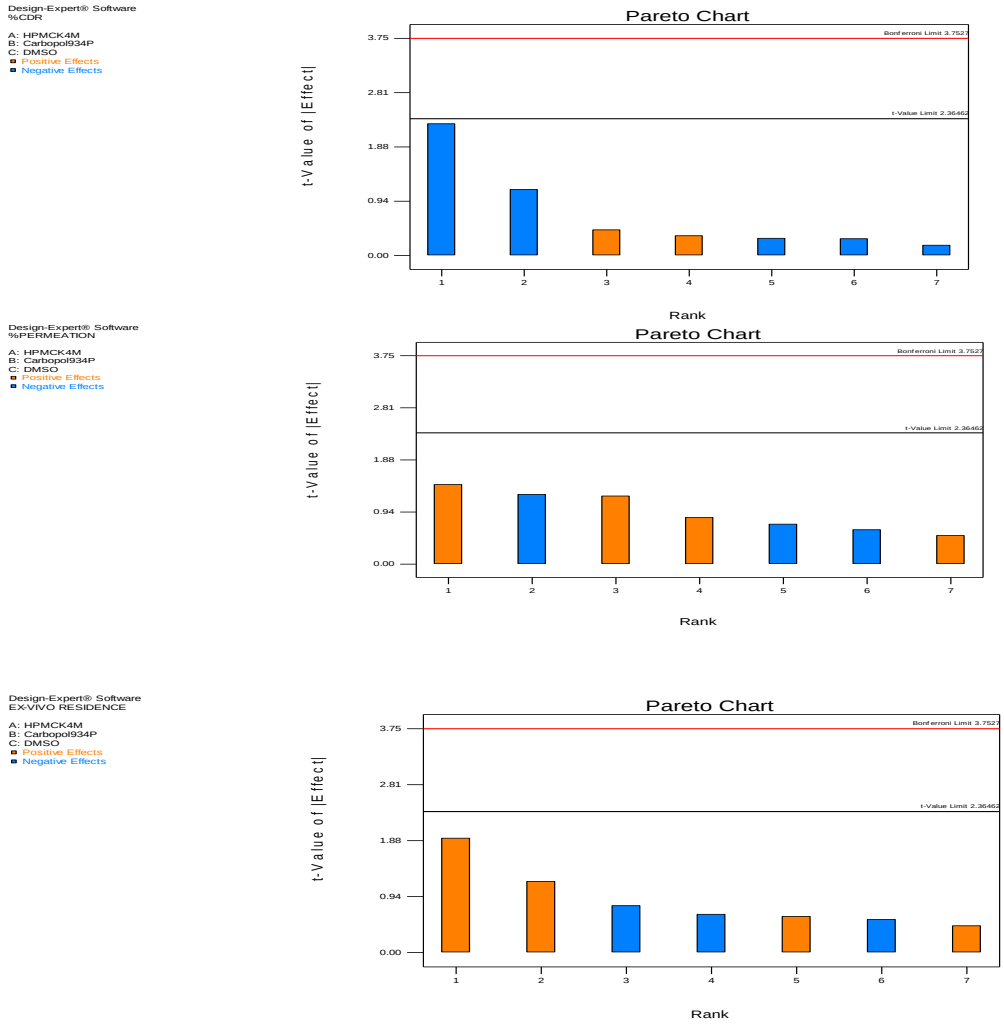
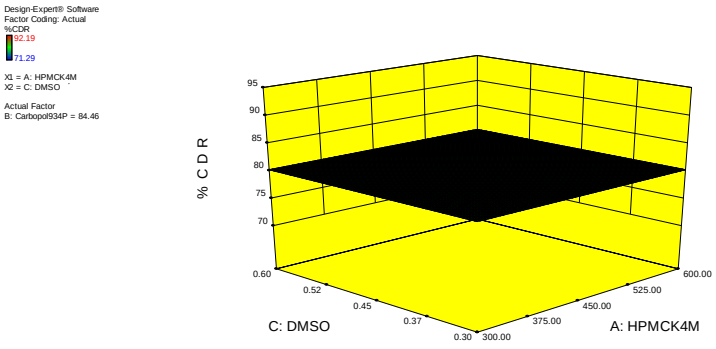
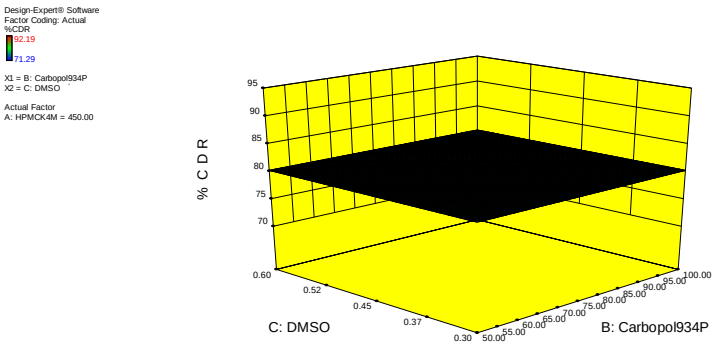


Fig 9.42: Response coefficient significant study on (a)%CDR at 8th hour (b)Cumulative %permeation at 8th hour (c)*Ex-vivo* residence time

Interactions studies and response surface plots:

The possible interactions between X1X2,X2X3,and X1X3 for each response were also investigated. The response surface plots generated using polynominal equations represent quantitative simultaneous effect of any two variables at constant level. The results were similar to interaction studies but were quantifiable. However Design Expert software can analyze both qualitative and quantitative effects of variables on the response parameters and hence can facilitative selection of optimized formulation.





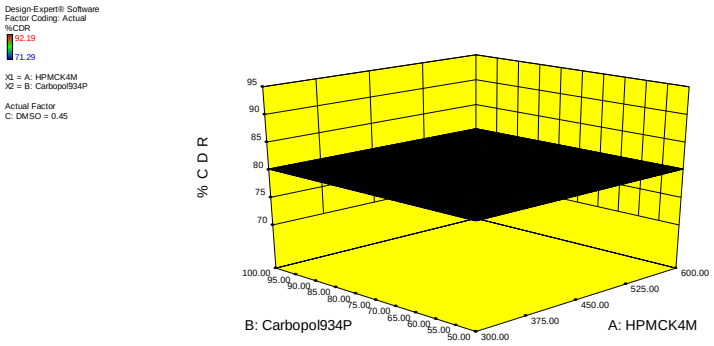
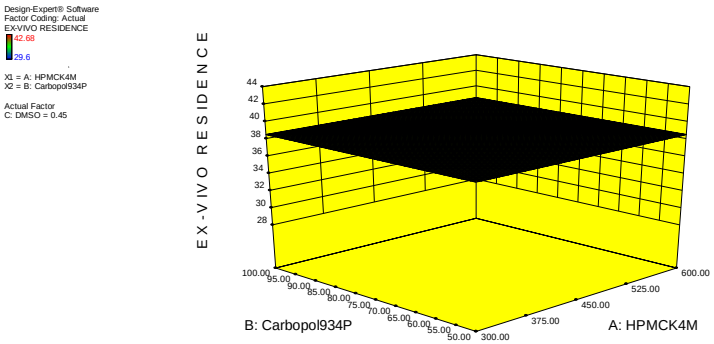
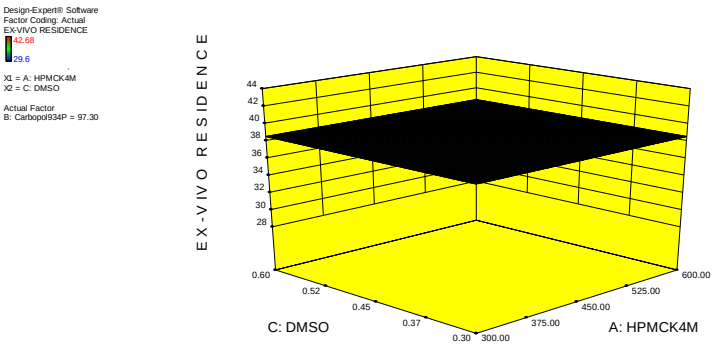


Fig 9.43: Response surface plots showing influence of independent variables on response parameter of buccoadhesive formulations %CDR at 8thhour





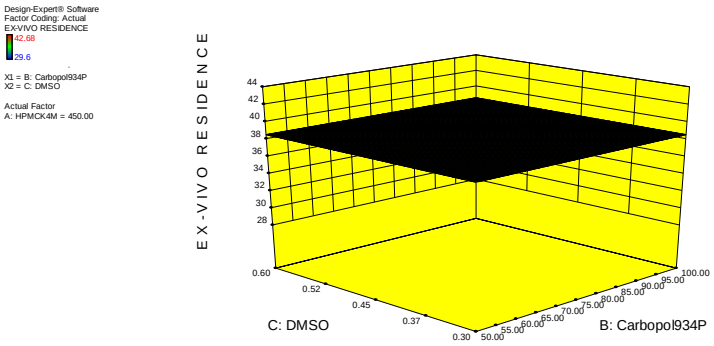
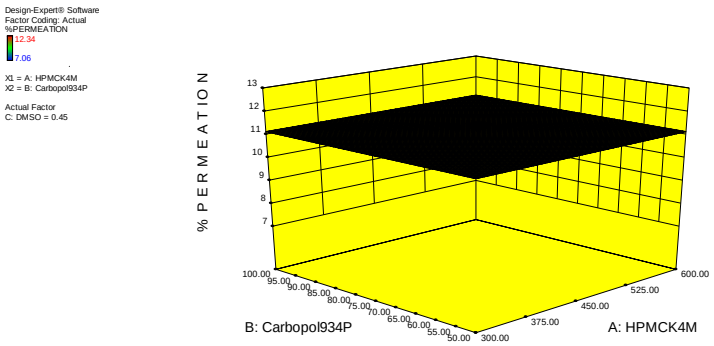
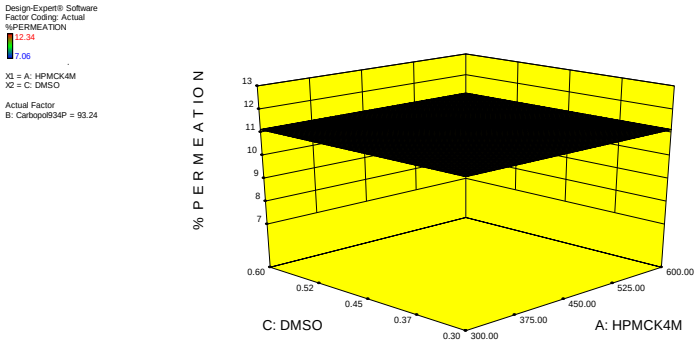


Fig 9.44: Response surface plots showing influence of independent variables on response parameter of buccoadhesive formulations ex-vivo residence time:





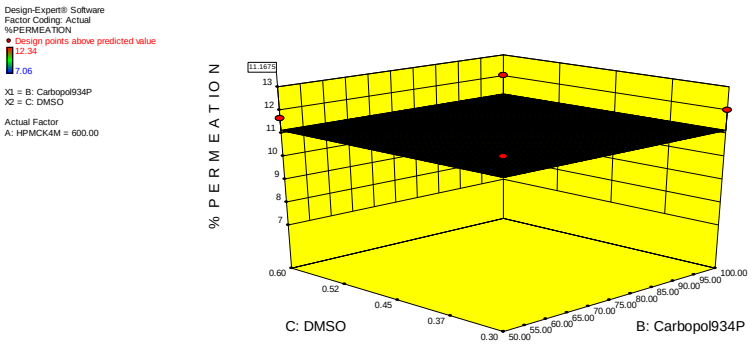


Fig 9.45: Response surface plots showing influence of independent variables on response parameter of buccoadhesive formulations cumulative %drug permeation at 8^{th}hour.

Selection of optimized formulation:

The qualitative and quantitative influence of independent variables on ex-vivo residence time, %permeability and %CDR were clearly interpreted from by design Expert that is an equally advantageous tool for selection of optimized formulation. The tools offer the possibility to vary each variable simultaneously and present optimum selections with their respective desirability value. According to our criteria of higher %CDR at 8th hour, higher residence time and higher cumulative %drug permeated after 8 hour, BF4 was selected as optimized formulation. Consequently ,the coded optimized level for the amount of HPMC K4M, concentration of Carbopol 934P and volume of DMSO for BF4 were identified as -1,+1,and+1 respectively.

9.3.14. Stability Study:-

After storage, the optimized formulation (BF4) was analyzed for various physical parameters; results are showed in Table 9.33.

Table 9.33: Stability studies of Buccoadhesive Film

Characteristic	Initials	1 Month	2 Month	3 Month
Appearance	White transparent film	No change	No change	No change
Surface pH	7.23 0.08	7.12±0.02	7.10±0.02	7.08±0.02
Ex-vivo residence time	11.81±0.57	11.28±1.028	11.28±0.01	11.26±0.02
***Ex-vivo* drug permeation at 8th hour**	42.68±1.35	40.93±0.64	40.87±0.52	40.32±0.22
Drug content	98.76± 0.28	97.74 ±0.57	97.37± 0.57	97.27± 0.65

*All the values are expressed as mean± SE, n=3.

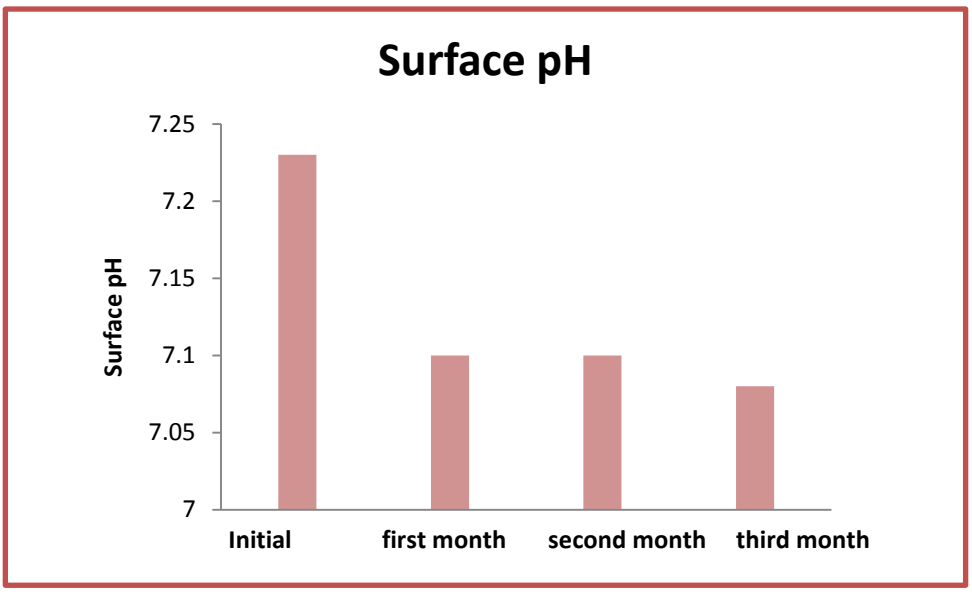


Fig. 9.46: Comparisons of Surface pH for formulation BF4 with initial and different periods of stability

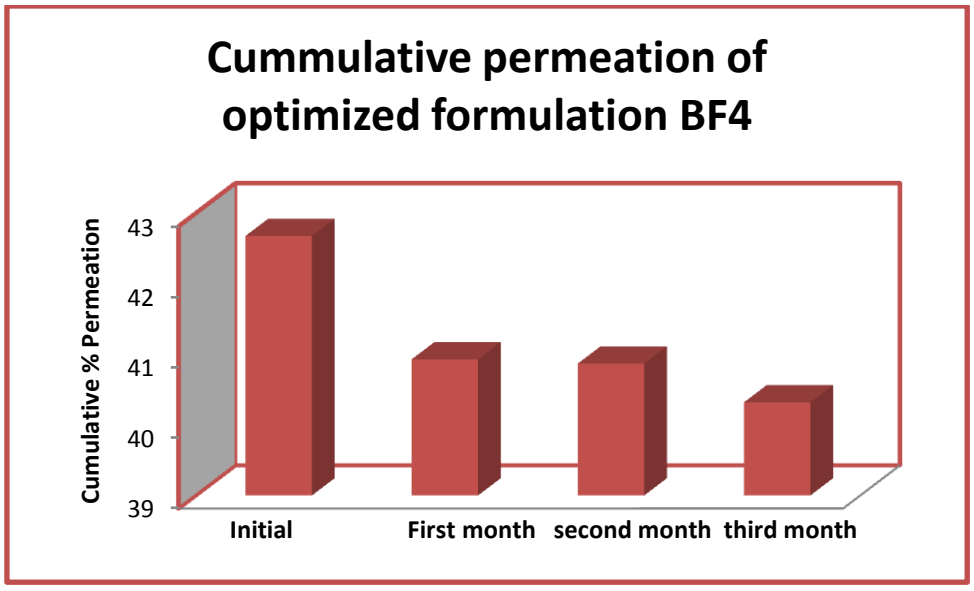


Fig. 9.47: Comparisons of Cumulative % drug permeation at 8th hours for formulation BF4 with initial and different periods of stability

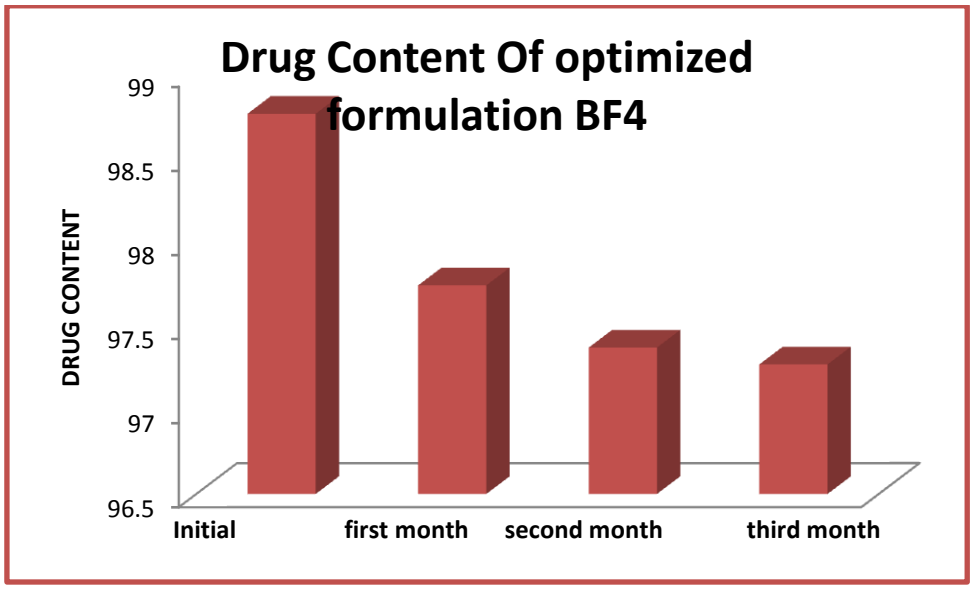


Fig. 9.48: Comparisons of Drug content for formulation BF4 with initial and different periods of stability

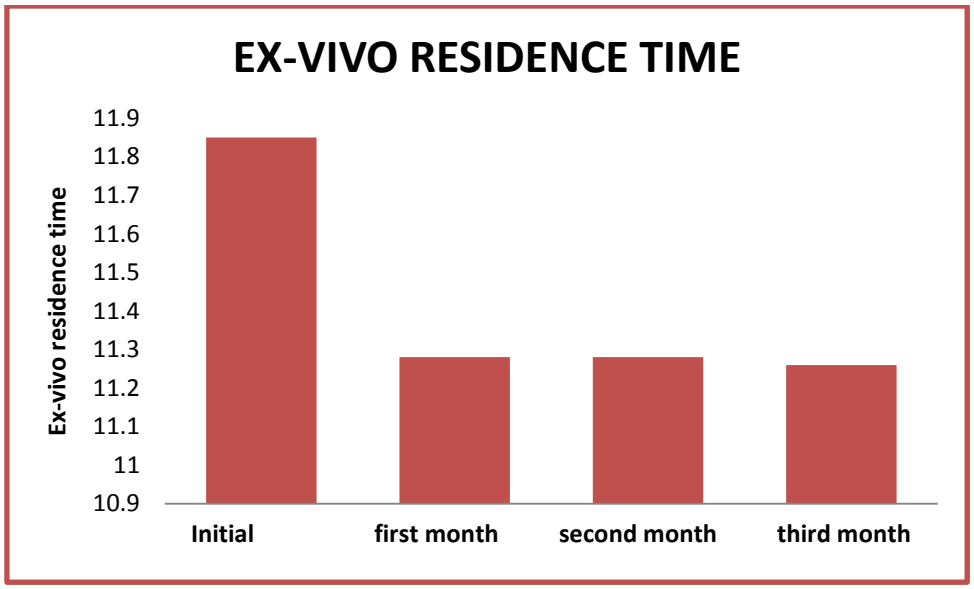


Fig. 9.49: Comparisons of Ex-vivo residence time for formulation BF4 with initial and different periods of stability

No major difference was found between evaluated parameters before and after storage and all are in acceptable limits. The Film showed satisfactory physical stability at 40°C at 75 % RH.

SUMMARY

AND

CONCLUSION

10. SUMMARY AND CONCLUSION

Metoprolol tartrate is a selective β-1 adrenergic antagonist used in the treatment of the cardiovascular system, especially Hypertension.

The present study was aimed to develop a new buccal mucoadhesive system for the delivery of Metoprolol tartrate. An attempt was made to formulate Metoprolol tartrate buccal film using new mucoadhesive polymers viz. Carbopol 934P, and HPMC K4M which have not been tried earlier with this drug.

Literature review on polymers strongly indicated that polymers selected for the present study have bioadhesive and matrix forming properties. Various formulations of mucoadhesive buccal Film of Metoprolol tartrate were prepared using various polymers in different proportions and combinations.

The initial part of work was started from the identification of drug. Identification of drug was determined by melting point, solubility and FTIR Study. The drug polymer interaction study was carried out by DSC study. So, it can be concluded that there is no interaction between drug and polymers used in the formulations.

The nine batches of buccal mucoadhesive films of metoprolol tartrate was prepared by solvent casting technique. These formulations were optimized using 2^3 factorial design model.

The formulated Film was evaluated for dimension thickness, weight, folding endurance. The observed and obtained information was in the acceptable limits. Folding endurance of the formulated films was flexible and displayed good folding endurance.

The swelling study of film was detailed that maximum degree of swelling was observed after 30min. Films containing high level of carbopol 934 P showed high degree of swelling; it was beneficial for buccal adhesion.

The Surface pH of film was found within the range were similar to that of pH of saliva in oral cavity.

The Ex-vivo buccoadhesive strength was performed and the values obtained were in the acceptable range except the formulation BF1.

The Residence time of all batches was studied and result showed that the mean adhesion time was increased in the formulation containing Carbopol 934P with HPMC K4M.

The data of *In-vitro* drug release study indicated that the formulation containing Carbopol 934P with HPMC K4M extended the release of the drug and these formulations also shown good bioadhesion on goat buccal mucosa. The BF1 and BF4 formulation was released the maximum drug at the 8^{th} hour but BF1 was rejected due to its less mucoadhesive strength. Hence, Formulation BF4 was the most promising formulation as it gives satisfactory drug release upto 8 hours and also produced more bioadhesive force as compare to other batch formulations.

The data of Ex-vivo buccal permeation study explained that the formulation containing high of DMSO showed higher permeation of drug in the range obtained with formulation BF4. The DMSO has been already reported as effective permeation enhancer.

The developed buccoadhesive film exhibited sufficient pharmacotechanical

Properties and buccoadhesive character. It was proved by sustaining the drug release of water soluble drug like metoprolol tartrate for 8 hr without causing any damage to the buccal mucosa. It was also confirmed by the histopathological studies.

The formulation BF4 had shown the satisfactory release of drug and excellent bioadhesive properties. The release data of drug was fitted with the kinetic modeling software in order to know the mechanism of drug release. It was found that the formulation BF4 follows higuchi model and diffusion mechanism.

The optimization of prepared films was calculated from design expert 8.0.2 software. The optimized formulation BF4 was subjected to stability studies, there was no appreciable change in the values during the 3 month period of study.

Hence, from the above information, it was observed that the formulation had feasibility of formulating buccal drug delivery in the form of buccal film of Metoprolol tartrate as; it can help to bypass extensive hepatic first pass metabolism and thus increasing efficacy of Metoprolol tartrate. Buccoadhesive film of Metoprolol tartrate was successfully developed to reduce the dosing frequency of the drug. The bioavailability of drug can also be improved with this buccoadhesive drug delivery system by avoiding extensive first pass effect, increasing efficacy, compliance and better clinical usefulness of patients.

An optimized formulation BF4 that has the potential to enhance the permeability limited bio availability and to provide a Unidirectional sustained drug delivery through the buccal mucosa.

Hence, from the overall inference, it can be concluded that the formulation BF4 was considered as best formulation.

FUTURE PROSPECTS

11. FUTURE PROSPECTS

The study requires attention of researcher to develop buccal drug delivery systems using other bioadhesive polymers and study its permeation through the membrane. Furthermore, the study can be extended to evaluate *in-vivo* performance and also *In-vitro-In-vivo* correlation of the film.

BIBLIOGRAPHY

12. BIBLIOGRAPHY

1. Agarwal S. P., Rajesh K. and Alka A. Preparation and evaluation of bioerodible Buccal tablets containing Clotrimazole. *Int. J. Pharm.*, **1996**, 138, 67-73.
2. Akbari J., Ali N., Djavad F., Massoud A., Mohammad R. S. and Majid S. Development and Evaluation of Buccoadhesive Propranolol Hydrochloride tablets formulations: effect of Fillers. *IL Framco.* **2004**, 59, 155-161.
3. Akpa P. A., Attama A. A., Onugwu L. E. and Lgwilo G. Novel buccoadhesive delivery system of Hydrochlorthizide formulated with Ethyl cellulose hydroxypropyl methylcellulose interpolymer complex. *Sci. Res. Essay.* **2008**, 3(6), 343-347.
4. Aleksandra M. S., Kinga C. and Tomasz C. Electrically enhanced and controlled drug delivery through buccal mucosa. *Acta Poloniae Pharmaceutica. Drug Res.*, **2008**, 65 (6), 767-769.
5. Anonymous. http:// www.google.com, A TO Z, drug index.
6. Anonymous. http:// www.google.com, drug bank
7. Anonymous. http://www.rxlist. Metoprolol tartrate.
8. Anonymous. Indian Pharmacopoeia. 5 th edn., Government of India, Ministry of Health and Family Welfare, The Indian Pharmacopoeial commission, Ghaziabad, **2007**, 1, 2, 179-183, 662-663, 1378 - 1380.
9. Ansel S. C., Allen L. V. and Popovich N. G. Pharmaceutical dosage form and drug delivery systems. 8th edn., Lippincott Williams & Wilkins, New York, **2009**, 234-238.

10. Anthony C., David M., Osselton and Brian W. Clark's. Analysis of drug and poisons. 3rd edn., Pharmaceutical press, London, **2004**, 2, 1271-1272.
11. Asha S. J., Sathesh B. P., Divakar G., Manoj K. J. and Kapil K. P. Development and evaluation of buccoadhesive drug delivery system for Atorvastatin Calcium. *J. Current Pharm. Res.,* **2010**, 1, 31-38.
12. Aulton, M.E. Pharmaceutics: The science of dosage form design. 3rd edn. Churchill Livingstone, New York, **2007**, 168-179, 461-464.
13. Ayyappan T. and Kasture P.V. Development and in vitro evaluation of a buccoadhesive Ondansetron Hydrochloride tablet formulation. *Indian Drugs*, **2005**, 43(2), 92-95.
14. Bandyopadhyay A. K. Novel drug delivery systems. 1st edn., Everest publishing house, Pune, **2008**, 143, 194-197.
15. Bankar G.S. and Rhodes C.T., Modern Pharmaceutics. 3rd edn. Marcel Dekker, New York, **1996**, 287-324, 668-669.
16. Bazlghak Abdul Rasool, Saeed A.Khan. In-viro Evaluation of Miconazole Mucoadhesive Buccal films.*IJAP.*,**2010,**2(4).
17. Becket A. H. and Stenlake J. B. Practical pharmaceutical chemistry. 4th edn., CBS. Publishers, New Delhi, II, **2005**, 278-280.
18. Belgamwar V. S., Shah V. H. and Surana S. J. Formulation and evaluation of oral mucoadhesive multiparticulate system containing Metoprolol Tartarate: an in-vitro characterization. *Int. J. Pharma. Excip*., **2007**, 14-23.
19. Bhalodia R., Basu B., Garla K., Joshi B. and Mehta K. Buccoadhesive drug delivery systems: A review. *Int. J. Pharm. Biosci.,* **2010**, 2, 1-23.

20. Brahmankar D.M. and Jaiswal S.B. Biopharmaceutis and Pharmacokinetics: A treatise. 2nd edn., Vallabh Prakashan, New Delhi, **2006**, 64-65, 335-357.
21. Cafaggi S., Leardi R., Parodi B., caviglioli G. and Bignardi G. Preparation and Evaluation of a Chitosan salt-poloxamer 407 based matrix for buccal drug delivery. *J. Control. Rel.,* **2005**, 102, 159-169.
22. Chatwal G. R. and Anand S. K. Instrumental methods of chemical analysis. Himalaya publishing house, Mumbai, **2007**, 2.747-2.750.
23. Chien Y. W. Novel drug delivery systems. 1st edn., Informa healthcare, New York, **2009**, 50, 139-140, 186, 197-199.
24. Chinna Reddy P,Chaitanya K.S.C.,Madhusudan Rao Y. A Review on Bioadhesive Buccal Drug Delivery Systems, current status of formulation and evaluation methods. *DARU*,**2011**,19(6).
25. Flavia Chiva Carvalho,Marcos Luciana Bruschi, Raul Cesar Evangelista, Maria Palmira Daflon Gremiao. Mucoadhesive Drug delivery systems. *BJPS,* **2010**, 46(1).
26. Gattani S. G., Patil S. B. and Wagh R. D. Mucoadhesive polymers: Means of improving drug delivery. *Pharma Times,* **2006**, 38(4), 25-28.
27. Ganesh Rajput,Falguni Majmudar,Jayvadan Patel. Formulation and Evaluation of Mucoadhesive Glipizide films.*Acta Pharm.***2011,**61;203-216.
28. Gaurav Kumar Sharma,Pramod Kumar Sharma,Mayank Bansal. A Review on Mucoadhesive Buccal Patch As a novel drug delivery system. *Pharmasm,* **2012,**3(2).
29. Gupta A., Garg S. and Khar R.K. Measurement of bioadhesive strength of mucoadhesive buccal Film: design of an *in vitro* assembly. *Indian Drugs*, **1992**, 50, 152-155.

30. Hirlekar R. S. Design of buccal drug delivery system for a poorly soluble drug. *Asian J. Pharm Clinical Res*., **2009**, 2 (3), 49-53.

31. Hung S. C. and Ahmad M. M. Design of a dissolution apparatus suitable for in situ release study of Triamcinolone Acetonide from bioadhesive buccal tablets. *Int. J. Pharm.,* **1995,** 121, 129-139.

32. Javed A., Roop K. K. and Alka A. Effect of polymers loading on drug release and bioadhesion of buccoadhesive carriers for local drug delivery of Triamcinolone Acetomide. *The Estern Pharmacist.*, **1999**, XL II (503), 115-119.

33. Johnston T. P., Montakarn C. and Nazila S. M. The use of mucoadhesive polymer in Buccal drug delivery. *Advanced Drug Delivery Reviews.* **2005**, 57, 1666-1691.

34. Lachmann L., Lieberman H. A. and Kanig J. L. The theory and practice of Industrial pharmacy. 3rd edn., Varghese Publishing House, Mumbai, **1987**; 67, 293-373.

35. Madhusudan R. Y., Vamshi V. Y., Chandrasekhar K. and Ramesh G. Development of Mucoadhesive Patches for Buccal Administration of Carvedilol. *Current Drug Delivery*, **2007**, 4, 27-39.

36. NagaRaju .K,Velmurugan. S,Deepila.B,Sundar vinu shitha.Formulation and *in-vitro* evaluation of buccal tablets of Metoprolol tartrate. *IJPPS*.**2011,**3(2);239-246.

37. Nagendra Kumar.D, S.B.Shirsand Para.M.S,and Makadia.C.K. Design and Evaluation of metoprolol tartrate containing Buccal tablets.*Dhaka Uni.J.Pharm.Sci.***2011,**10(2);101-108.

38. Nakhat P. D., Babla I. B., Rathi L. G., Yeole P. G. and Ghule B. V. Design and characterization of buccoadhesive Film of Promethazine Hydrochloride. *Indian Drugs,* **2007**, 44 (7), 520-528.

39. Navneet G., Arya1 R. K., Akanksha G. and Neetesh N. J. Development and evaluation of mucoadhesive buccal Film of Salbutamol Sulphate. *Int. J. Pharm. Pharmaceut. Sci.,* **2010**, 2 (2), 40-43.

40. Parvez, N., Ahuja A. and Khar R.K. Development and evaluation of mucoadhesive buccal film of Lignocaine Hydrochloride. *Indian J. Pharm. Sci.,* **2002**, 64(6), 553-567.

41. Pankaj Kumar, Gulshan Chabra,and Kamla Pathak. Development and Statistical Optimization of Buccoadhsive films of Amiloride Hydrochloride;*in-vitro* and *ex-vivo* Evaluation. *Ind J Pharm Edu Res,* **2012.,** 2(46), 145-153.

42. Patel V. M., Prajapati B. G., Patel H. V. and Patel K. M. Formulation, evaluation and comparison of bilayered and multilayered mucoadhesive buccal devices of Propranolol Hydrochloride. *AAPS Pharm. Sci. Tech.,* **2007**, 8 (1), 1- 8.

43. Peppas N. A., Laura S. and Jose D. Design of poly (ethylene glycol) - tethered copolymers as novel mucoadhesive drug delivery systems. *Euro. J. Pharm. Biopharm.,* **2006**, 63, 11-18.

44. Poptani Sanjay D, Gohel mukesh C, Parikh Rajesh K, Patel vipul P. Prepration and Evaluation of Osmatic controlled Drug Delivery System of Metoprolol tartrate. *Int.Bull.Drug Research.,***2012,**1(1); 84-93.

45. Prachiti P. V., Aswini R. M., Sanjay J. K. and Komal V. I. Buccal drug delivery system. *Int. J. Pharm. Excip.,* **2006**, 33-38.

46. Prakash Raob,and Gandhi Purvesh.Formulation and Evaluation of Mucoadhesive Buccal drug delivery system of Metoprolol tartrate by using central composite Design.*RJPS,* **2011,**1(2),146-157.

47. Pramodkumar T. M. and Shivakumar H.G. Novel core in cup buccoadhesive systems and films of Terbutaline Sulphate–development and in vitro evaluation. *Asian J. Pharm Clinical Res*., **2006**, 1 (3-4), 175-187.

48. Rajesh S. P. and Poddar S. S. Development and Characterization of Mucoadhesive Buccal Patches of Salbutamol Sulphate. *Current Drug Delivery*, **2009**, 6, 140-144.

49. Rama Bukka, Kalyani prakasam,Chintan D Patel. Preparation and Evaluation of Intra oral Drug delivery System for Rosagiline mesylate. *IJPSDR.*,**2010,** 2(3),294-301.

50. Ramana M. V., Nagda C. and Himaja M. Design and evaluation of mucoadhesive buccal drug delivery systems containing Metoprolol Tartarate. *Indian J. Pharm. Sci.*, **2007**, 69(4), 515-518.

51. Ravikumar Reddy J, Indira muzib Y, Formulation and Evaluation of mucoadhesive Buccal film of Amiloride Hydrochloride. *IGTPS.,* **2012,**3(3);828-837.

52. Rathbone M.J., Ponchel, G. and Ghazali F.A. Oral mucosal drug delivery. 1st edn., Marcel Dekker, New York, **1996**, 52-54, 241-242.

53. Ravi Saurabh,Rishabha Malviya and pramod Kumar Sharma.Trends in Buccal film;Formulation Characteristics,Recent Studies and Patents. *European J.APPL.SCI.,* **2011**, 3(3);93-101.

54. Raymond C. Rowe, Paul J. Sheskey and Sian C. Owen. Hand book of Pharmaceutical Excipients. 4 th edn, pharmaceutical press, London, **2003**, 89, 132. 297, 323.

55. Robert M. Silverstein. Spectrometric identification of organic compounds, 6th edn., **2003**, 71-144.

56. Sachin Shandar Lokhande,Sandele S Lahoti. Buccoadhesive Drug Delivery System Need.*Aj bio pharm,***2012,**2(14);29-36.

57. Saroj Kumar R. and Bala P. Bioadhesive Polymeric Platforms for Transmucosal Drug Delivery Systems – a Review. *Trop. J. Pharm. Res.,* **2010**, 9 (1), 91-104.

58. Shojaei A.H. Buccal mucosa as a route for systemic drug delivery: A review. *J. Pharm. Pharmaceut. Sci.*, **1998**, 1(1), 15-50.

59. Siddarth S. D. and Upendra K. Design and development of Felodipine buccal mucoadhesive patches. *Int. J. Current Pharm. Res.*, **2010**, 2(3), 70-75.

60. Sinko P.J. Martin's Physical Pharmacy and Pharmaceutical Sciences. 5th edn., Lippincott Williams and Wilkins, New York, **2006**, 637-638.

61. Sudhakar Y., Kuotsu K. and Bandyopadyyay A. K. Buccal bioadhesive drug delivery- a promising option for orally less efficient drugs: Review. *J. Control. Rel.,* **2006**,114, 15-40.

62. Suraj P. A., Alka A. and Monica D. Development of buccal Film of Diltiazim Hydrochloride. *Int. J. Pharm. Sci.,* **1995**, 57 (1), 26-50.

63. Surendar Verma, Mahima Kaul, Aruna Rawat and Sapha Saini. An overview on buccal drug delivery system. *IJPSR*.,**2011**;2(6):1303-1321.

64. Thimmasetty J., Pandey G. S. and Sathesh P. R. Design and *in vivo* evaluation of Carvedilol buccal mucoadhesive patches. *Pak. J. Pharm. Sci.* **2008**, 21(3), 241-248.

65. Tortora G.J. and Grabowski S. R. Principles of anatomy and physiology. 10th edn., John Wiley and Sons, New York, **2003**, 858-859.

66. Tripathi K. D. Essentials of Medical Pharmacology. 5th edn., Jaypee Brothers, New Delhi, **2004**, 602 – 604.

67. Venkatalashmi R, Yajaman Sudhakar, Madhuchudana Chetty C,and Mohan Varma M.Buccal drug Delivery from metoprolol tartrate polymeric mucoadhesive film.,*JPR*,**2011,**4(11),3892-3896.

68. Venkatalashmi R, Yajaman Sudhakar, Madhuchudana Chetty C,and Mohan Varma M. Buccaldrug Delivery using Adhesive Polymeric Patches., *IJPSR*, **2012,** 3(1);35-41.

69. Vyas S. and Khar R. K. Controlled Drug Delivery Concepts and Advances In: Systems for Colon Specific Drug Delivery. Vallabh Prakashan, New Delhi, 1st edn., **2002**, 292-294.